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Page 19, line 1, *read* 38% *for* 38.

Page 107, paragraph 1, lines 1, 3, and 4, *change* macrocarpa *to* macrospora.

Page 111, title, line 4, *read December for January.*

Page 142, line 5, *change* about 80 per cent *to* above 70 per cent.

Pages 369, 371, 373, 375, 377, 379, 381, 383, 387, 389, running heads, *read* stripe *for* barley.

Page 395, paragraph 2, line 4, *change* $\sigma^2_{A-B} = \sigma^2_A + \sigma^2$ *to* $\sigma^2_{A-B} = \sigma^2_A + \sigma^2_B$.

Page 408, table title, *change* of the uredospores *to* to the uredospores.

Page 411, table 10, *change* 5 c.c. N/10 in 100 c.c. *to* 5 c.c. N/10 NaOH in 100 c.c.

Page 414, paragraph 5, line 2, *read* pentathionates *for* penathionates.

Page 415, reference 6, *read* e *for* ed.

Page 416, reference 27, *read* Polythionaten *for* Polythionen.

Page 431, footnote, line 2, *after* Pathology *insert* and Agricultural Chemistry.

Page 480, paragraph 1, line 7, *read Heliothrips for Haliothrips;* line 8, *read vaporariorum* for *vaporarium.*

Pages 506 and 507, *interchange* entire figure legends.

Page 520, paragraph 5, line 4, *change* high (30° C.) *to* at a high temperature (30° C.).

Page 663, paragraph 2, line 9, *after* Zehner *add* and Humphrey.

Page 668, reference 9, *after* Zehner, M. G., *add* and Harry B. Humphrey.

Page 679, line 7, *read* Bensaude *for* Bensuade.

Page 680, reference 3, *read* racines *for* racine.

Page 689, line 1, *change* A. Lange Norbert *to* Norbert A. Lange.

Page 703, item 4 under *Philippine organism, change* three *to* two.

Page 704, paragraph 3, *change* three *to* two.

Page 843, line 4, *read* from *for* form.

Page 844, paragraph 2, line 3, *change* figure 3, *1, to* figure 2, *1.*

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Page 926, reference 5, *before* ..... *insert* Burrill, T. J.

Page 948, line 6, *change* 4 *to* 3; line 7, *change* 5 *to* 4.

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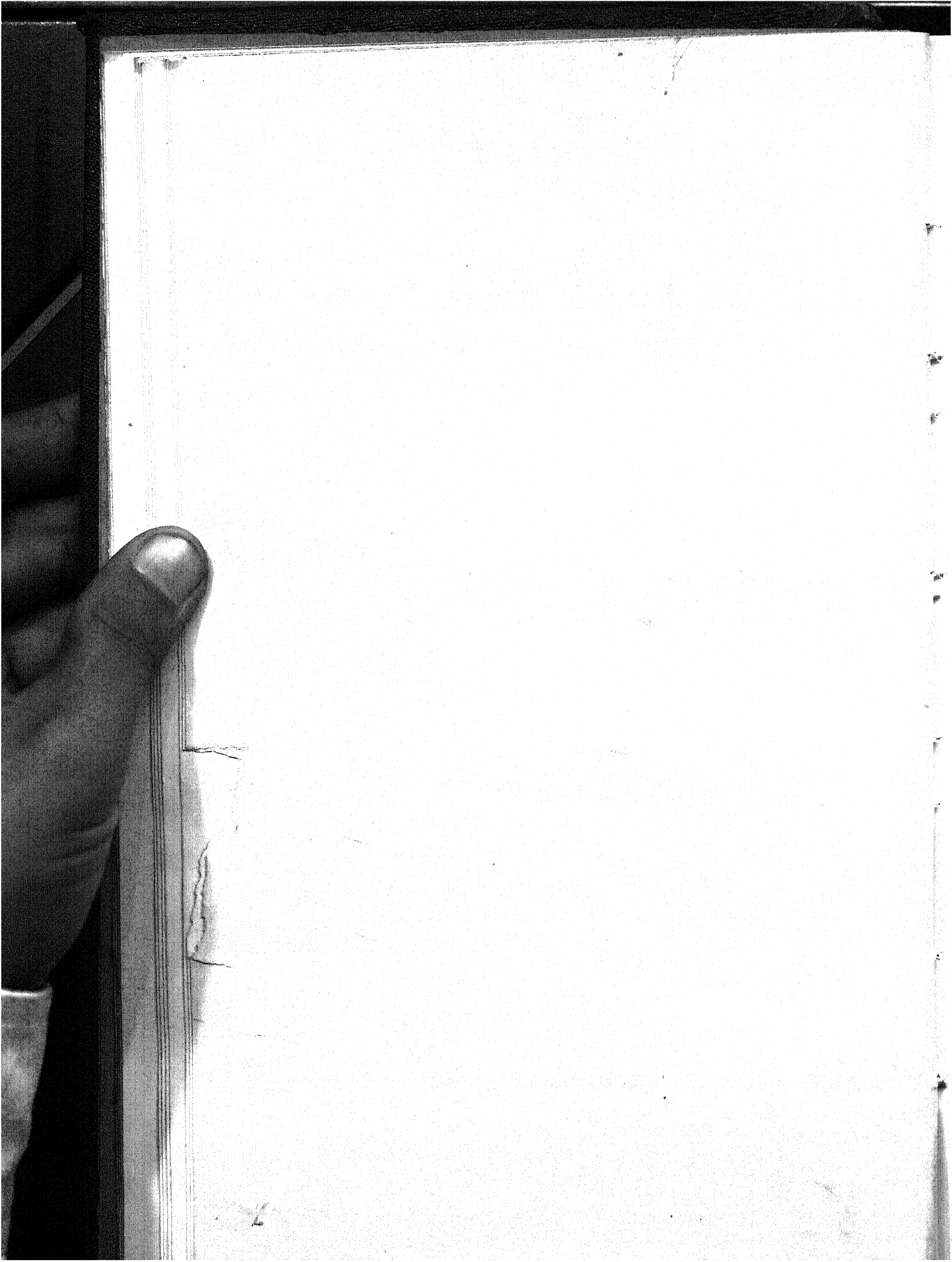
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THE GENUS PHYTOMONAS

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Unlike the fungous plant pathogenes, found in a great many families and genera scattered throughout the three main groups of the fungi, the bacteria which cause diseases in plants are restricted to a rather limited group in the Eubacteriales. The large number of known species of these bacterial pathogenes is continuously increasing, but the genera to which they belong in the various systems of classification are few. Briefly described, the bacterial plant pathogenes are nonspore-forming aërobic, motile or nonmotile rods. Other restrictions could be placed on the group that would limit it still further, but the description would become highly involved and not very helpful. The above simple description suffices for our purposes, and perhaps some objections to it could be brought forth from the literature on the subject. These objections, however, are very few and at times open to some doubt. *Bacillus mesentericus* as a plant pathogene is an outstanding example, but even this spore-forming organism causes only a tuber rot and not a disease of the growing plant. The brief description of the plant pathogenes as given above is practically correct and covers those forms dealt with in this article.

With such a small group of organisms to consider, one naturally would be of the opinion that little difficulty would be experienced with its taxonomy. In this case, however, the reverse is true. A confusion exists here that is not equalled in any one group of the fungi engaging the interest of plant pathologists. This confusion, too, is not of recent occurrence, but has existed for some time and is now of almost everyday unconcern. Everyone recognizes it, but no one does anything about it. It is at present, to a considerable extent, treated with levity.

This confusion in the nomenclature of the bacterial plant pathogenes is due to the general use by the plant pathologists of various systems of classification. There are several of these systems each of which is recognized and the investigator in bacterial diseases of plants merely expresses a preference for one of them. An example of this may be cited. A new bacterial pathogene is described and given a binomial name, but a footnote frequently will be found stating the system of classification followed and one or two

other generic names which could be used if other classification were employed. Here a preference is shown for one of the systems in use, but the author's allegiance is more than likely to shift in his next article. Also, some authors have refused to make a decision and have used generic names from various classifications. Since several of these generic names occur interchangeably in the various systems, further confusion has arisen and plant pathogenes are recognized now only by their specific name.

The systems of classification in use at the present time by the plant pathologists are those of Migula, Lehmann and Neumann, Erwin F. Smith, and Bergey. The order of the systems given above is more or less chronological. The confusion, or the general use of all four classifications by the plant pathologist, has not come about through differences in basic principles. These principles are practically the same for three of these systems used, and for the fourth, Lehmann and Neumann's, no classification or divisions and subdivisions exist for the plant pathogenes. It appears inexcusable that we should have arrived at such a state of chaos. The reason for having done so can be comprehended only by examining the history of these systems and their adoption by the plant pathologist.

In examining these systems of classification and the extent of their use, one is forced to separate the plant pathologist from the bacteriologist. This is a rather lamentable fact, but nevertheless true. The plant pathologist has drifted away from the science of bacteriology for many years until now there is little contact between the two groups. On the other hand, we have been closely associated with the mycologist with advantages on both sides. The disadvantages in our separation from the bacteriologist are apparent. This separation of the two groups is emphasized here from the fact that each has regarded differently the systems of classification proposed for bacteria.

The true bacteriologist has not been so hampered with systems as has the plant pathologist; in fact, he has, as a rule, paid little attention to them. In the last two decades of the nineteenth century and the beginning of the twentieth, when the spirit of the time was that of classifying and experimenting with classification, Migula's and Lehmann and Neumann's classifications were followed by the bacteriologist. Later, certain features of each of these systems were employed. For instance, the Pseudomonas group (Migula), that is, the rod forms having polar flagella, was recognized, as was also the Bacillus group (Lehmann and Neumann), the forms producing spores. Further recognization of other groups arose and, in the United States, the present drift in nomenclature of the bacteria has, in general, crystallized itself into the Bergey classification. This no doubt will be modified, since complaints are arising on all sides concerning certain of the

genera. The system does show, however, the direction in which the bacteriologists are going in this field.

The American plant pathologists, on the other hand, at first accepted Migula's system, possibly because Erwin F. Smith did. Chester's volume on Determinative Bacteriology also helped to establish this system in America. Lehmann and Neumann's system was and is still more or less unknown to the American plant pathologists. The European plant pathologists, however, especially those on the Continent, follow Lehmann and Neumann, so one is forced to recognize this system in the literature. In 1906, Erwin F. Smith brought forth his classification and, although it was never considered by the bacteriologist, it was immediately adopted by the plant pathologist. There were reasons for this. The plant pathologists were introduced to bacteriology by Erwin F. Smith, and his fight to establish the fact that bacteria can cause diseases in plants is one of the high points in this field. E. F. Smith was preëminent in plant pathology and it was but natural that they should follow his lead in nomenclature. Furthermore, the plant pathologists were all schooled in taxonomic mycology, a subject which at that time was greatly concerned with priority, and Smith's classification was based on priority. No new concepts were expressed. The three systems with the same generic names applied to different groups were now in continual use, and confusion arose. To get away from this state of affairs, a few adopted Bergey's system when it arrived. Entirely new generic names were applied here.

In showing a preference for one of the systems of classification it would be reasonable to expect an investigator to examine the basis for each classification. Perhaps this is being done, but a discussion of this phase of the subject, as far as I know, has not appeared in literature. And, since the basis for classification is of primary importance, it seems pertinent to review it here. Migula used the presence and absence and mode of attachment of flagella as generic characters in his classification. The genus Bacterium is composed of nonmotile forms; Bacillus, of forms motile by peritrichous flagella; and the genus Pseudomonas is composed of rods which are motile by means of polar flagella. When Erwin F. Smith presented his classification of bacteria in 1906, he still considered the presence and absence and the position of flagella as sound generic characters, but digressed from Migula in the following manner. He held that the generic name Bacterium had priority over Pseudomonas, since Cohn (1872) had first used it for the rod forms having polar flagella. This consequently left the nonmotile forms without a name; so Aplanobacter was coined for this genus. Smith was not the only one to point out the priority of the name Bacterium over Pseudomonas. Vuillemin (1913) independently reached the same conclusion.

Since at that time (1906) a starting point for nomenclature for bacteria had not been agreed upon, Smith's arguments are logical if one accepts his basis for generic characters.

Nevertheless, due to the fact that the term Pseudomonas was probably so well established, bacteriologists failed to regard these disclosures with interest. The situation also was further complicated by the increasingly large group of workers in bacteriology who held that the presence and absence of spores were of generic significance and that flagella were of minor importance. Consequently, for them, Smith's or Vuillemin's conclusions would have no bearing on change of generic names. It was upon this basis that Lehmann and Neumann had presented their system of classification, namely, the presence or absence of spores. In the genus Bacillus they placed all rod forms producing spores. The genus Bacterium was composed of those which did not produce spores. It may be seen readily that all plant pathogenes would fall in this last genus.

A most radical digression from the systems of classification just described occurs in that of Bergey, in that all the plant pathogenes are placed in a separate tribe, Erwinieae. The first step in this direction had appeared in the Final Report of the Committee of the Society of American Bacteriologists on Characterization and Classification of Bacterial Types, when the tribe Erwinieae was proposed for the plant pathogenes in the family Bacteriaceae. Bergey, however, went further and made a new genus, Phytomonas, composed of motile plant pathogenes from the genus Pseudomonas of the Committee's report, together with the nonmotile plant pathogenes. The new genus, Phytomonas, was then placed in the tribe Erwinieae, next to the genus Erwinia. This tribe is based entirely upon the ability of its members to cause disease in plants, and all other characters of the bacteria are disregarded. The reason for the separation of the plant pathogenes was not discussed and it seems evident that the close relationship of the genus Erwinia to the coli group, and of certain members of the genus Phytomonas to the fluorescent group was overlooked or not known. In the general classification of bacteria, Bergey does not always use the character of pathogenicity to separate groups. He does follow this system in the families Bacteriaceae and Coccaceae, but not in the families Bacilliaceae and Spirillaceae. In the Actinomycetes he has allowed certain of the forms pathogenic to plants to associate with animal pathogenes and saprophytes. He is not entirely consistent.

The use of pathogenicity as a character of such importance as to separate tribes and genera should be considered closely before adoption. It has never been employed in the main groups of fungi. The close relationship of many members of the genus Erwinia to *Escherichia coli* Escher-

ich has been mentioned. *Phytomonas marginale* Brown can scarcely be distinguished from *Pseudomonas aeruginosa* Schröter. Nonpathogenic forms of *Phytomonas tumefaciens* Smith and Townsend are evidently numerous in the soil, and, so far as Bergey's classification goes are at present, homeless. Furthermore, pathogenicity is in many bacteria a very delicate character and may be lost quickly when the organism is grown in pure culture. The same thing probably occurs in nature under adverse conditions.

A system of classification for bacteria seeking to be a natural system should be workable as well, and primary characteristics should be considered first. Many plant pathogenes are not always found associated with their host plant. In isolating an organism under such circumstances the character of pathogenicity would have to be disregarded from the very nature of the case. Only a plant pathologist searching for a definite species would think of making inoculation tests. One would not know what plant out of hundreds to use. The test would be unthought of until many of the characteristics were determined and the organism appeared to be a certain pathogene. By that time, alas, its pathogenicity might have been lost. Rahn (1929) in his article, "Contributions to the classification of Bacteria," has emphasized this point under the title of "Practical Impossibilities in Taxonomy."

In considering the subdivisions in the tribe Erwinieae of Bergey, one finds that his separation of the two genera is based primarily on the presence and absence and position of flagella. He only digresses from Migula and from Smith in that he places the nonmotile pathogenes in the same genus with the forms possessing polar flagella. This move has proved a little disconcerting to the plant pathologist since it has been felt by some that the nonmotile forms are a distinct group. The separation of the genus Erwinia on the character of peritrichous flagella is orthodox and probably justifiable; at least, there is no indication to the contrary. The genus is a well-recognized group and each species possesses this character.

ANALYSIS OF THE GENUS PHYTOMONAS

Having pointed out the defects of Bergey's classification, I still follow it because of its clarity and efficiency. One is not required to use a footnote. Furthermore, plant pathology is too interdependent on the other sciences to become isolated, especially in matters of taxonomy. We must look to the systematist in bacteriology just as we do in mycology or phanerogamic botany, although in this case the bacteriologists have treated us rather shabbily. We can, however, call attention to defects in systems of classification and, furthermore, point out methods of construction for bettering these systems. The remainder of this article is such an attempt.

It is felt that the basis for classification of the bacterial plant pathogenes was established when the number of the pathogenes was small and our information concerning them was meager. The number of known species is now many, considerably over a hundred, and it is time to take stock of what knowledge we have of these forms. In this article I do not intend to examine all the bacterial pathogenes, but to confine myself only to one genus of the Bergey classification. This genus, Phytomonas, is chosen for the following reasons: First, it contains by far the larger number of species of the two genera of the tribe Erwinieae and appears to be a rather heterogeneous mixture. The question arises whether or not it can be considered as one genus. Also, the genus combines the nonmotile forms with the motile forms having polar flagella, and it should be determined whether or not this is justifiable. And third, having worked with a number of rather dissimilar species within the group, I feel more familiar with this genus. The genus Erwinia, which is not considered in this article, is composed of few species and appears to be a fairly well-defined group.

The ideal method in studying and analyzing a genus is to obtain all the species and grow them in pure culture under identical conditions. There is, however, one great difficulty, namely, that but a very small percentage of these species could be secured at present. Consequently, another method was sought and the one used here is that set forth by Rahn (1916), which is based on the assumption that in any well-defined group many of the primary characteristics are identical. Rahn has demonstrated that certain correlations exist in the natural groups and has pointed out those characters on which separation and divisions may be based. He used the description of species given by Migula and by Lehmann and Neumann as the basis of his work. In the following work with the plant pathogenes the procedure employed by Rahn has been followed more or less.

In following this method of analysis one primary weakness arises. One is forced to accept the descriptions of species of a great many investigators where the technique varies not only with the time but with the different laboratories. Frequently this has been overcome, however, when several persons have worked on the same organism, and a composite picture from the various descriptions can be drawn.

Bergey in the second edition of his "Manual of Determinative Bacteriology" lists 39 species of Phytomonas.[1] In table 1, I have listed 77 species, and this is not entirely complete. For certain species, the names of which appear in literature, I have been unable to find a description and have omitted them. This type of omission, however, is but occasional.

[1] The third edition of this manual will contain descriptions of many more species of this genus.

Then, there were several species which, although described as of this genus, apparently do not belong there. As an example, I may mention *Phytomonas dissolvens* Rosen which, with the exception of its polar flagella, stained by a method as yet not divulged, has more characteristics of the Erwinia group. This staining method, too, would place *E. amylovora* (Burrill) Com. S. A. B. in the genus Phytomonas, but it has been omitted in this table.

Varieties have not been added to the list since it was felt that they would disturb the ratios of characters. Most varieties have been described mainly on pathogenicity and not on cultural characteristics. From another point of view, it is true that some of these omitted varieties are as important pathologically as or more so than the original species. *Phytomonas phaseoli* var. *sojense* (Erw. Smith) Hedges, *Phyt. medicaginia* var. *phaseolicola* (Sackett) Burkholder and the several varieties of *Phyt. translucens* Smith, Jones and Reddy might be mentioned. It is likely that certain species listed in table 1 are synonyms or only varieties; that is, they differ only in pathogenicity. *Phytomonas sojae* Wolf and *Phyt. stizolobii* Wolf probably belong to this case. *Phytomonas rhizoctonia* Thomas has been avoided in the table since it is felt that, like *Phyt. stizolobii,* under certain conditions it might be motile. At least this organism is quite different from the other nonmotile forms. *Phytomonas viridifaciens* Tis. and Will. has been considered a synonym of *Phyt. vignae* Gar. and Ken.; *Phyt. citrareifaciens* Lee and *Phyt. cituputeale* C. O. Smith have been shown by Miss Bryan (1928) to be identical with *Phyt. syringae* van Hall. There appear to be some differences in the descriptions of the pathogene as given by the three authors, but this may be due merely to technique or to fluctuating characters. Miss Bryan has done a commendable piece of work and more like it is needed. Due to a footnote in Gardner and Kendrick's article, "The Bacterial Spot of Tomato" (1921), a supposition has crept into phytopathological literature that *Phyt. exitosa* Gar. and Ken. is a synonym of *Phyt. vesicatoria* Doidge. The organisms differ in reaction to the Gram stain and in sugar fermentations, and these appear to be sufficient to separate them as distinct species.

In a search through the literature it was rather astonishing to find what few characters had been determined for some of our fairly common species. Outside of its pathogenicity, we know very little concerning *Phyt. juglandis* Pierce. *Phytomonas phaseoli* (Erw. Smith) Bergey et al. was named in 1898, but it was not until Rapp (1920) and later Hedges (1924 and 1926) worked on the species that we had an adequate description of it. The description of *Phyt. beticola* (Smith, Brown and Townsend) Bergey et al. given in 1911 is strikingly different from that given by Miss Brown in 1928. In



TABLE 1.—*Giving characters of 77 members of the genus Phytomonas*[a]

Phytomonas	Agar pig.	Mot.	Gram	Lit. milk				Star.	Dex.	Suc.	Lac.	Gel.	Nit.	Ind.	H_2S	Aër.	C.
				Curd	Ac.	Alk.	Pep.										
alboprecipitans	–	+	–			+	+	+	–	–	–	–	+	–	–	+	+
agropyri	yellow	–	W+		+W		–	+W	+	+	+	–	–	–		+	+
amaranthi	yellow	+	–			+	–	+				–		–		+	
andropogoni	–	+										–	–			+	
angulata	green	+	–			+	+	–	+	+	–	+	–	+		+	–
apii	–	+	–			+	+	–	+	+	–	+	–	–		+	
aptata	green	+	–			+	+	–	+	+	–	+	–	+	–	+	–
atrofaciens	green	+	–			+	+	+W	+	+	–	+	–	+W	+W	+	+
avenae	–	+	–	curd	+		–	–	+	+	+	+	+	–		+	
barkeri	–	+	+			+	+	+W	–	–	–	+	–	–			–
beticola	yellow	+	W+			+	+	+W	+	+		+	+	–	+	+	+
bowlesii	green	+	–	curd		+	+		+	–	–	+	+	+	+	+	–
campestre	yellow	+	–			+	+	+	–	–	–	+	–	+	+	+	–
cerasi	green	+	–					–	+	+	–	+	–			+	
cannae	–	+	–			+	+	+W	–	–	–	+	+	–	+	+	+
cichori	?	+	–					–				–	–	–			
citri	yellow	+	–			+	+	+	+	+	–	+	–	–		+	
coronafaciens	–	+	–	S. curd		+	+	+W	+	+	–	+	–	–	–	F	+
cucurbitae	yellow	+	–	curd			+	+W				+	–				

TABLE 1—(*Continued*)

Phytomonas	Agar pig.	Mot.	Gram	Lit. milk				Star.	Dex.	Suc.	Lac.	Gel.	Nit.	Ind.	H_2S	Aër.	C.
				Curd	Ac.	Alk.	Pep.										
delphinii	green	+	–			+	+	+W	+	+	–	+	–	–	–	+	+
destructans	–	+	+					+	+	+	+	+	+			F	
erodii	green	+	–		+		+		+	+	+	+	–	+	–	+	
exitosa	yellow	+	–		+		+	+	–	–	–	+	–				
flaccumfaciens	yellow	+	+		+		+W		+	+	+	+W	–	–	–	+	
glycinea	–	+	–	curd		+	+	–	+	+	–	–	–	+		?	+
gummisudans	yellow	+	–	S. curd	+W		+	+W	+	+	–	+	–	–	+	+	+
hibisci	–	+	–				+					+	+W	–		+	
hyacinthi	yellow	+	–			+	+	+	+	+	–	+	–	+W	+	+	
holci	green	+	–				+	–	+	+	–	+	+	–		+	–
insidiosa	yellow	–	+	S. curd	+		–	+	+	+	+	+	–	–	--	+	+
intybi	?	+	–					–				+	+	–			
juglandis	yellow	+						+				+				+	
lachrymans	green	+	–			+	+	–	+	+	–	+	–	+W	–	+	+
maculicola	–	+	–			+	–	+W	–	–	–	+	–	+W	–	+	
malvaceara	yellow	+	–	curd		+	+	+	–	–	–	+W		–		+	–
marginale	green	+	–	S. curd			+	+W	+	+	–	+	+	–	–	+	+
marginata	–	+	–	S. curd	+		+	–	+	+	+	+	–	+W	+W	+	+
martyniae	green	+	–	S. curd	+W		+	–	+	+	–	+	+	–	+	F	+

TABLE 1—(*Continued*)

Phytomonas	Agar pig.	Mot.	Gram	Lit. milk				Star.	Dex.	Suc.	Lac.	Gel.	Nit.	Ind.	H_2S	Aër.	C.
				Curd	Ac.	Alk.	Pep.										
matthiolae	–	+	–	curd	+		–					+			–	+	
medicaginis	green	+	–			+	–	–	–	–	–	–	–	–	–	+	
mellea	yellow	+	–	curd		+	+	–	–	–	–	+	–	–	–	F	+
michiganensis	yellow	–	+	curd	+W		–	+W	+	+	+	+W	–	–		+	
mori	–	+	–			+	+	+W				–	–	–		+	–
nectarophila	green	+	–				+		+	–	–	–	–	–		F	+
panici	–	+	–			+	+	+	–	–	–	+	+	–	+	+	+
papulans	–	+	–	S. curd		+	+		+	+	–	+				+	–
pelargoni	–	+	–	S. curd		+	+	+W	–	–	–	+	–	+W	+	+	+
phaseoli	yellow	+	–			+	+	+	–	–	–	+	–	–	–	+	
pisi	–	+	–	S. curd		+	+	–	+	+	–	+	–	–	–	+	–
proteamaculans	yellow	+	+	S. curd	+		+	+W	+	+	–	+				F	–
pruni	yellow	+	–	S. curd			+	+W	+	+	+	+	±	+W		+	+
peurariae	green	+	–			+	+	+W	+	+	–	+	–	–	–	+	–
pseudozoögloeae	yellow	+		curd	+		–		+	+	+	+					
rathayi	yellow	–	+		+		–	+W	+	+	+	+	–			+	+
rubrilineans	yellow	+	–			+	+	–?	+	–	–	+	+	–	–	F	–
savastonoi	–	+	–			+	–	+W	+	+	–	–	–	+W		+	

TABLE 1—(*Continued*)

Phytomonas	Agar pig.	Mot.	Gram	Lit. milk				Star.	Dex.	Suc.	Lac.	Gel.	Nit.	Ind.	H_2S	Aër.	C.
				Curd	Ac.	Alk.	Pep.										
semina	–	+	+			+	+	–	+	+	–	+	–			F	+
sojae	–	+	–			+	–	+W	–	–	–	–	–	–		+	+
solonaceara	–	+	–			+	+	–	–	–	–	±	+	–		+	–
solaniolens	yellow	+			+		–	+W	–	–	–	–	–				
stewarti	yellow	–	–		+		–	+W	+	+	+	–	–	–	–	+	
stizolobii	–	+	–			+	–	–	–	–	–	–	–	–	–	+	+
striafaciens	–	+	–	S. curd		+	+	+	+	+	–	+	+W	–	+	+	+
syringae	green	+	–	S. curd		+	+	+W	+	+	–	+	–	–	–	F	–
tabaca	–	+	–			+	–	–	+	+	–	+	–	–		+	–
teutlia	–	–	+				–			+		–	–				–
tolaasii	green	+	–	curd		+	+	–	+	–	–	+	+	+W		F	–
translucens	yellow	+	–	curd	+		+	–	+	+	+	+	–	+W		+	+
trifoliora	green	+	–			+	–	+W	+	+	–	–	–			+	+
tritici	yellow	+					–		+		+W	–	+				
tumefaciens	–	+	–	curd		+W	–	+W	+	+	–	–	–	+W	+	+	
vasculara	yellow	+	±			+	–	+W	–	–	–	±	–	–		+	
vesicatoria	yellow	+	+		+		+	+W	+	+	+	+	–	–		+	+
vignae	green	+	–			+	+	–	+	+	–	+	–	–	–	+	

TABLE 1—(*Continued*)

Phytomonas	Agar pig.	Mot.	Gram	Lit. milk				Star.	Dex.	Suc.	Lac.	Gel.	Nit.	Ind.	H_2S	Aër.	C.
				Curd	Ac.	Alk.	Pep.										
viridilivida	green	+	–	curd		+	+	–	–	–	–	+	–	+	+	+	+
vitians	yellow	+	–			+	+	+W			–	+	–	+W			
woodsii	?	+				+	–					–	–				

[a] A brief explanation of the above table follows. Under "Agar pig.," pigment production is listed on agar only. A dash (–) designates a white or colorless form; yellow is any shade from a cream yellow to a deep yellow; and green refers to the fluorescent pigment produced and dissolved in the media. Mot. is motile and Gram refers to the Gram stain. Under milk there are four columns: Curd; S. for soft; Ac. for acid; Alk. for alkaline; and Pep. for peptonization. With reference to peptonization it should be stated that descriptions as a rule have reported milk cleared (+) or not cleared (–), so there may be some doubt here. Star. stands for starch and plus denotes that it is digested; W, if weakly so. Under the three sugars listed (+) means that acid is produced; Gel. is an abbreviation for gelatin and Nit. for nitrates. A plus under the last-named denotes that they are reduced to nitrites. Ind. stands for Indol production and is followed by H_2S production. A plus under Aër. means an aërobe, and F a facultative anaërobe. C. refers to capsule production.

examining table 1 a number of forms may be found for which we know little concerning their cultural reactions. Too many species have been described on the basis of pathogenicity to some host, with a few cultural and morphological characteristics which are slightly more than sufficient to place them in a genus. Work of this type simply lays foundations for synonyms. It would be much better for all concerned, and especially for future students, if a sufficient number of characters should be determined to compare the organism with other members of its genus and cross inoculations made to ascertain whether it is a new form or an old form on a new host.

The description of the characters of many species, too, has been vague. In many cases, especially in the sugar broths, all the shades of Ridgway and all the degrees of cloudiness and turbidity have been noticed, without considering that these no doubt would vary with temperature, hydrogen-ion concentration, and other factors not mentioned. There are certain instances in which the most or only important point, namely, whether or not a given sugar was fermented, is not stated. In preparing table 1, I have been forced at times to make a guess concerning certain characters. In the list of titles appended to this paper one may easily find where my data have been obtained.

Sixteen characters were used in this table of species. They comprise all but one of the primary characteristics and a few of the secondary ones listed in the descriptive chart for 1924 prepared by the Committee on Bacprimary characteristic omitted is that of spore formation: This character teriological Technic of the Society of American Bacteriologists. The one does not exist among the bacterial plant pathogenes. The table is valuable in showing what we know at present concerning the characteristics of the species of this genus. Further work is needed in filling up the gaps and working over certain of the characters. In many instances the cultural methods used by various authors in dealing with the sugars would fail to disclose a small amount of acid produced.

In table 2 the methods of Rahn are followed, and the percentages of the characteristics are given for the entire genus and for various groups in this genus. While there are 77 species dealt with, the percentages are seldom based on this number but on that of species on which information is given on the characteristic under consideration. This varies and is usually somewhat short of 77. Consequently a definite series of percentages is not to be expected in the table. The figures in the group column refer to the number of species found in that group. All other figures in the table are percentages.

Table 2 is large and consists of many figures. To point out all correlations and lack of such would require space and would only be confusing.

TABLE 2.—*Percentage distribution of characters in various groups of species within the genus Phytomonas*[a]

Group	Pig.	Mot.	Gram	Milk			Star.	Dex.	Suc.	Lac.	Gel.	Nit.	Ind.	H_2S	Aër.	Cap.
				Acid	Alk.	Pep.										
Total 77	Y 38 G 25 – 37	92	18	25	75	69	64	72	67	22	76	25	30	40	83	61
Nonmotile 6	Y 83 – 17	–	83	100	0	0	100	100	100	100	50	0	0	0	100	75
Motile 71	Y 33 G 28 – 39	+	11	21	79	75	61	69	62	16	77	28	31	42	82	60
Gram + 13	Y 69 – 31	61	+	63	37	50	91	83	91	63	84	16	0	33	72	66
Green fl. 19	–	100	0	14	86	88	38W	94	77	6	83	27	48	25	80	50
Colorless 28	–	100	15	12	88	65	56	59	61	13	68	30	32	50	88	61
Yellow 28	–	82	33	54	46	63	84	68	62	42	81	17	25	45	87	62
Yellow 13	–	100	8	31	69	85	85	38	31	0	92	9	30	57	75	37
Pep. + 49	Y 35 G 30 – 35	100	10	19	81	+	64	71	63	13	91	28	36	44	80	60
Pep. – 22	Y 48 G 9 – 43	77	33	47	53	–	76	66	66	40	41	10	21	14	100	75

TABLE 2.—(*Continued*)

Group	Pig.	Mot.	Gram	Milk			Star.	Dex.	Suc.	Lac.	Gel.	Nit.	Ind.	H_2S	Aër.	Cap.
				Acid	Alk.	Pep.										
Star. +	Y 69	92	16	18	82	82	+	45	45	18	82	30	20	50	91	66
14	– 31															
Star. +W	Y 46	85	29	32	68	60	+W	69	69	24	75	14	30	50	91	75
27	G 25															
	– 29															
Star. –	Y 9	100	4	16	84	80	–	80	75	30	83	32	38	36	80	53
23	G 43															
	– 48															
Sugar +++	Y 71	65	54	92	8	70	72	+	+	+	86	36	36	20	91	100
14	G 7															
	– 22															
Sugar ++–	Y 15	100	7	12	88	92	54	+	+	–	85	16	38	40	81	60
27	G 37															
	– 48															
Sugar –––	Y 44	100	12	11	89	66	77	–	–	–	72	22	25	45	93	70
18	G 6															
	– 50															
Gel. +	Y 40	95	19	27	73	83	62	75	67	23	+	30	32	41	82	60
58	G 26															
	– 33															
Gel. –	Y 30	84	13	23	77	19	71	61	53	23	–	11	23	20	92	77
19	G 17															
	– 53															
Nit. +	Y 23	100	12	18	82	87	57	75	57	26	94	+	27	75	75	61
18	G 31															
	– 46															

TABLE 2.—(*Continued*)

Group	Pig.	Mot.	Gram	Milk			Star.	Dex.	Suc.	Lac.	Gel.	Nit.	Ind.	H_2S	Aër.	Cap.
				Acid	Alk.	Pep.										
Nit. – 52	Y 38 G 26 – 36	88	20	27	73	65	63	69	69	19	69	–	38	29	88	66
Ind. + 19	Y 26 G 37 – 37	100	0	11	89	84	43	76	65	21	84	16	+	63	89	61
Ind. – 41	Y 38 G 24 – 38	90	19	27	73	72	66	66	61	20	75	33	–	25	84	63
H_2S + 14	Y 36 G 21 – 43	100	7	21	79	93	84	64	61	8	93	43	57	+	93	84
H_2S – 21	Y 28 G 43 – 28	90	10	25	75	71	59	70	65	20	81	15	20	–	81	61
Fac. An. 10	Y 30 G 40 – 30	100	60	25	75	100	50	90	60	10	90	44	28	20	–	55
Cap. + 28	Y 36 G 25 – 39	89	18	28	72	78	70	71	64	26	75	28	32	55	78	+
Cap. – 18	Y 24 G 41 – 35	94	17	6	94	89	50	75	59	0	89	31	33	28	75	–

[a] All figures in the table are percentages with the exception of those in the first column, where they refer to the actual number of species in each group.

Furthermore, upon examining the table closely all these correlations appear to be the result of the occurrence of several well-defined groups which appear almost intact in a number of the divisions listed in table 2. These definite groups will be pointed out and emphasized. First, however, one should look at the genus and note the general lack of correlations of characteristics which exist in it as a whole. Outside of motility and the reaction to the Gram stain, the percentages are neither high nor low. Most anything may be expected within the genus. In other words, it does not constitute a well-defined group of organisms. The reason for this confusion, one may assume, is the fact that the genus is composed of several natural groups, and that order may be obtained only by determining what they are. One conclusion which might be arrived at hastily is that the combination of the nonmotile with these motile forms has probably caused a disturbance. There are, however, only 6 of these nonmotile species listed in table 1, and these hardly could be expected to change the percentages to any great extent. Nevertheless, it will be seen in table 2 that these six species are a very closely related group of pathogenes; more so than one would expect. Should they, then, have been placed with the motile species? From examining them as a separate nonmotile group, one would say probably not; but their removal does not appear to improve matters, since it may be seen that the percentages of the 71 motile species do not deviate to any extent from the percentages of the entire genus. It still remains a group of many rather unrelated types. The question then arises whether any of the motile species are related to this nonmotile group. A search through table 1 reveals the fact that certain forms do exist which, but for their character of motility, fit in very nicely. The two most outstanding species of this group are the yellow forms, *Phyt. flaccumfaciens* Hedges and *Phyt. vesicatoria* Doidge. Besides these, several other less closely related forms are to be found, but the addition of these to the group would not materially lessen the high percentage of the majority of characters. Disregarding these outlying forms, however, one finds here eight species which can be considered the center or nucleus of a natural group of bacteria, but a group which can not be defined entirely on the absence of flagella. The Gram reaction, the ability to ferment lactose, and the ability to produce a yellow pigment are also important characters. No one character can be used to differentiate the group, and for convenience it might be referred to as the *Stewarti* group from one of its earliest described species. An intensive cultural study of these forms, no doubt, would produce results of importance.

The next character used to differentiate groups in table 2 is that of pigment formation. This is a character which has been used before by various investigators to separate genera of bacteria but with varying amounts of

success. In the genus Phytomonas there are forms producing a yellow pigment, others a green flourescence, and still others which are nonchromogenic. An attempt has been made to separate the 77 species listed in table 1 on this basis. There have been described 28 yellow bacterial pathogenes, but when these are placed together in table 2 it may be seen that they are in no sense a natural group. If one considers, however, that the *Stewarti* group constitutes approximately one third of these chromogenic forms, their heterogeneity is not surprising. If, however, one removes from the 28 yellow species those forms which ferment lactose and those the descriptions of which are so limited as to make work with them difficult, 13 species remain. These are listed in table 2 and appear to be a natural group, but one not so well-defined as the *Stewarti* group. This new group could be characterized as composed of species which are yellow motile forms that are Gram-negative, active in peptonizing milk, liquefying gelatin, and digesting starch, but are not nitrate reducers. They do not ferment lactose, but may or may not ferment dextrose and saccharose. One possible error in the table is that 31 per cent of the species produce acid in milk, a character one expects to find associated with lactose fermenters. This error might be due to the preparation of the milk and the formation of some of the lower sugars through decomposition in the process of sterilization. Further working over some of the other characteristics also might raise their percentage, since a number of these species were described when our technique was considerably less adequate than it is today. In referring to this group in the remainder of this article the name *Campestre* will be applied, since this organism is representative of these 13 yellow species.

In examining both the *Campestre* and the *Stewarti* groups, one will find that there will be some difficulty in separating them along a definite line of cleavage. There appear to exist some intermediate types between the two. Rahn (1929) has pointed out that such forms occur among the bacteria and has dwelt upon the confusion that they cause in taxonomy. *Phytomonas beticola, Phyt. pruni* (Erw. Smith) Bergey et al. and *Phyt. translucens* are, no doubt, such intermediate forms between the *Campestre* and the *Stewarti* groups. They probably have a closer relationship with the latter but, if placed there, would have a tendency to lower the high percentages of certain of the characters. On the other hand, the disturbance would be even greater in the *Campestre* group. At present these species probably should remain as intermediate forms and no attempt be made to place them in one or the other of the two groups.

A further group which appears in table 2 is that of the green fluorescent forms. To any one who has worked with any of these pathogenes this is not a surprise. The similarity of these bacteria has been noted before. In table 2 they do not appear so closely related as the members of the *Stewarti* group. Nevertheless, they are a fairly well-defined group and the percentages might

appear higher if they were better understood. For instance, in table 2, 38 are shown to digest starch. This process in the green fluorescent species is a very weak one and the percentage from one point of view should approach zero. Also, it is to be noted particularly that certain forms of the green fluorescent types like *Phytomonas erodii* Lewis upset the correlations considerably, and the group would be closer knit if such forms were dropped. Eighteen species are listed here which produce a green fluorescent pigment, but there are others in this group not brought out in these tables. Chromogenesis in these studies has been determined on agar cultures, and it is known that certain of the green fluorescent types will not produce this pigment on beef extract agar, the agar usually employed by investigators. Furthermore, this ability to produce pigment is frequently lost in pure culture. For these two reasons it is felt that a number of species belonging to the group may be found with the white or colorless forms. If it were possible to find a definite medium on or in which the character of green fluorescent pigment production could always be determined, the group could be limited more readily. Uschinsky's solution might be such a medium; at least, it appears so from superficial observation.

If one eliminates from the genus Phytomonas the three groups just discussed there remain a number of colorless forms with which very little can be done. These species contain, no doubt, a number of green fluorescent bacteria, as suggested in the foregoing. Their ability to produce the pigment is possibly very weak, which accounts for its being overlooked. Also, many other of these colorless forms have a limited description, and, consequently, they are difficult to work with statistically. Too few of their characteristics are known. The best explanation to offer concerning these remaining forms of the genus Phytomonas is that a colorless group exists. This is reasonable to expect. The group, however, is not unlike the green fluorescent group in many respects, and a number of intermediate forms between the two are to be found. Statistically, this is rather difficult to prove with our present knowledge, but an examination of the colorless forms listed in table 1 points to this belief. Further work is necessary, however, to show this to be true.

The three and possible four bacterial groups existing in the genus Phytomonas would, if it were not for the character of pathogenicity, find places in several genera of the family Bacteriaceae of Bergey. The *Stewarti* and the *Campestre* groups would fall in the genus Flavobacterium, since they are both composed of species forming a yellow pigment. As has been pointed out in this article, however, the ability of these organisms to produce a yellow pigment does not appear to be a character on which to base a natural group. As far as known, no intensive work has been done on the genus Flavobacterium and it is not known whether or not it could be separated into two groups as the yellow plant pathogenes have been.

In a similar manner, the green fluorescent group of plant pathogenes would fall in the genus Pseudomonas of Bergey.[2] It is well known that many of the nonpathogenic fluorescent types are ubiquitous and are common saprophytes found living in the soil, on seeds, and on various parts of plants. In this there appears to be a relationship in habitat between the pathogenes and the saprophytes. This fact also might at least indicate the possible origin of the green fluorescent pathogenes which have arisen so suddenly and mysteriously at times.

The colorless plant pathogenes would fall into the genus Achromobacter.

One character of interest to the plant pathologist not touched upon here and possibly of great help in this work is the type of symptom produced on the plant by the pathogene. It is known that certain closely allied organisms produce similar diseases in animals. The members of the genus Pasteurella are an example of this. Similar cases might occur among the plant pathogenes. This character, however, would probably be difficult to point out since in certain bacterial diseases of plants the causal organism may produce various types of symptoms. It is a matter, nevertheless, worthy of consideration.

CONCLUSIONS

While this study has not presented any definite conclusions, it is hoped that bringing together the material and presenting it to the plant pathologist will make more apparent the difficulties and needs in this field. The article has pointed out, however, the reasons for the confusion that exists in the taxonomy of the bacterial plant pathogenes and the fault that investigators are making by looking at the subject over antiquated systems of classification and not examining the group as a whole. It has also demonstrated that certain natural groups do exist and that there probably are others which will come to light after more exact study. That these groups can be defined on single characteristics, as presence or absence of flagella, appears to be doubtful. What ranking these groups should receive can be determined only by further work. The question, too, arises as to whether the plant pathogenes should be treated separately or whether they should not find their places in the genera of the bacteria as a whole. Proof is yet to be given that pathogenicity is a character on which to separate genera, let alone one on which a tribe may be formed.

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[2] The genus Pseudomonas of Bergey is entirely distinct from the genus Pseudomonas of Migula. The former is characterized by the ability of its members to produce a green fluorescent pigment. The latter genus is based on the character of polar flagella possession.

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"FERN-LEAF" OF TOMATO[1]

N. MOGENDORFF

The symptom known as "fern-leaf" of tomatoes is chiefly characterized by a filiform abnormality of part of the foliage. For many years this condition has been considered as associated with a virus and as such it has often been referred to by pathologists as a symptom of ordinary tomato mosaic. At the same time, however, the common occurrence of tomato mosaic and the only sporadic appearance of fern-leaf together formed a problem which has never been fully solved. More recently it has been claimed by some investigators that fern-leaf symptoms on tomatoes may not necessarily be the result of a virus in the present sense of the word, but that these may be produced by a substance which has the nature of a toxin (22). It has also been suggested in a recent paper that fern-leaf symptoms may, in some cases at least, be of an hereditary nature (23).

The present investigation was undertaken with the purpose of determining, as far as possible, the factors governing the development of fern-leaf symptoms as resulting from virus infections. The evidence secured by Johnson (17) that fern-leaf is often associated with cucumber mosaic on tomatoes was taken as a base for further studies. Johnson was not able, however, to produce fern-leaf symptoms at will with this virus; consequently it appeared that some other factors were involved. In the present paper it will be shown that these factors are connected with the method of infection, the age of the plant at the time of infection, and with certain environmental conditions.

Since Clinton (8) carried out his cross-inoculation experiments with the tobacco-mosaic virus on tobacco and tomato, it has generally been assumed that the virus causing tomato mosaic is identical with the ordinary tobacco-mosaic virus. It was, therefore, considered advisable to study simultaneously the tobacco-mosaic disease of tomatoes for comparative purposes. This paper is consequently concerned with two virus diseases of the tomato, namely, tomato mosaic as caused by *Tobacco virus 1* (18) and tomato mosaic as caused by *Cucumber virus 1* (18). For convenience these diseases will be referred to as tobaco mosaic on tomato and cucumber mosaic on tomato.

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The author wishes to acknowledge his indebtedness to Dr. James Johnson, under whose guidance this work was done, for his valuable advice and suggestions; to Dr. L. R. Jones for helpful criticism; and to Dr. I. A. Hoggan for aid in preparing the manuscript.

REVIEW OF LITERATURE

Mosaic symptoms on tomatoes were first described in 1902 by Woods (31) on plants which he had accidentally infected with tobacco-mosaic virus. It was not until 1907, however, that Clinton (8) in Connecticut reported an "infectious chlorosis" in field tomatoes, which was found to be "of the same nature as calico of tobacco." A tomato-mosaic disease occurring in greenhouses in Holland was studied by Westerdijk (30). Although she was not able to transfer this disease to tobacco, it has been assumed by some that she was dealing with ordinary tomato mosaic, while others expressed doubt about its character. The tomato-mosaic disease reported from Massachusetts by Stone (28) and later by Chapman (5) has also been interpreted as identical with tobacco mosaic. A description of symptoms produced on tomatoes by artificial inoculation with tobacco-mosaic virus was given by Allard (1). Gardner and Kendrick (12) made an extensive study of tomato mosaic as it occurs in Indiana, but they give little evidence for the identity of the disease. From their descriptions it would appear that at times more than one virus may have been involved in their studies. The descriptions of tomato mosaic as given by Neal and Barker (26), Chupp (7), and others are essentially the same as those reported by Gardner and Kendrick (12).

Tomato fern-leaf as a symptom of tomato mosaic was first described by Westerdijk (30), who states that this symptom was never observed as a result of natural infection in the field or greenhouse, but only on artificially inoculated plants. Humbert (14) states that at times string-leaf or fern-leaf was observed in Ohio on mosaic tomatoes. McCubbin (24) calls it "a symptom of somewhat rare occurrence", which is associated with tomato mosaic, while McKay (25) states that filiform leaves are unusual on mosaic tomatoes, though not rare. Berkeley (3) reports from Canada that a filiform and spindly leaf is often associated with tomato mosaic. From the literature it thus appears that "tomato mosaic" generally is considered to be identical with tobacco mosaic. Its symptoms answer, in general, the descriptions given by Allard (1) and Gardner and Kendrick (12), while fern-leaf is commonly accepted as a symptom that is frequently associated with this desease.

In more recent years, symptoms differing from those referred to above have been reported, with the result that some investigators have commenced to recognize distinct types of mosaic symptoms on tomatoes, without asserting, however, that these are caused by different viruses. For instance, Bewley (4) distinguishes five main types of mosaic symptoms on tomato foliage among which he lists the "tendril type", which is characterized by a reduction of the lamina and the production of tendril-like leaves. These various symptoms may, according to this investigator, be found

singly on individual plants or together on the same plant. Although granting that a considerable amount of investigation is still necessary, Bewley (4) is inclined to believe that "these symptoms are manifestations of the same disease, their appearance being governed by a number of factors, chief of which are the environmental conditions under which the host plant is grown, and the degree of resistance exhibited by it." Weber and Ramsey (29), working in Florida, recognize three types of tomato mosaic, namely, the linear type with filiform leaves, the crinkle type, and the mottle type. The linear type is reported as rather uncommon, while the crinkle type is by far the most common type of tomato mosaic in Florida. Apparently on the basis of symptoms, Poole (27) distinguishes three virus diseases on the tomato, namely, streak, mosaic, and the filiform disease. Johnson (18) reports eleven virus diseases affecting the tomato, which he obtained by inoculation with eleven distinct viruses. This would point to the possibility of great complications in the symptomatology of tomato-virus diseases and might explain some of the existing confusion.

MATERIALS AND METHODS

The experimental work was carried out in the University of Wisconsin greenhouses at Madison, Wisconsin, between the months of September and June. The plants were started in flats in a warm greenhouse at 28–32° C. and transplanted into compost soil in four-inch pots. The common tomato (Marglobe variety) was used in the majority of the experiments, but in some cases the "potato-leaved" or large-leaved variety, Hudson Valley Maid (*Lycopersicum esculentum* Mill. var. *grandifolium* B.), served as host plant.

The minimum number of plants inoculated for each treatment was five, and in many cases units of ten plants were used. Each unit was accompanied by at least two and usually four or five noninoculated control plants. Great care was taken to use healthy plants of the same age and of uniform size. The plants ordinarily were inoculated by the cotton-needle method when they were about 5 cm. high, at the time when the third leaf (exclusive of the seed leaves) was just emerging.

Since tobacco is a better source of inoculum than tomato, the inoculum was prepared by pressing the crushed, fresh leaves of cucumber-mosaic or tobacco-mosaic tobacco through a piece of cheesecloth. The expressed juice was used for inoculation directly after extraction. The tobacco-mosaic virus used for inoculum was collected in the field and identified as *Tobacco virus 1* (18). The cucumber-mosaic virus was kindly furnished by Dr. Doolittle of the U. S. Department of Agriculture; this virus answered the description of *Cucumber virus 1* (18). If young tobacco plants, kept under greenhouse conditions at 30° C., are inoculated with

Tobacco virus 1 and with *Cucumber virus 1,* it is easy for the trained eye to distinguish between the two viruses, judging by symptoms only. Therefore, after inoculating each series of tomatoes, five tobacco plants were inoculated with the same inoculum, in order to check the identity and purity as well as the virulence of the virus employed.

EXPERIMENTAL RESULTS

After preliminary experiments had corroborated Johnson's (17) findings concerning the association between fern-leaf symptoms and cucumber mosaic on tomato, special efforts were made to study the symptomatology of this disease. A fair percentage of infection was usually obtained in these preliminary experiments with *Cucumber virus 1*, but only very few or none of the infected plants would show typical fern-leaf symptoms. In most cases the disease revealed itself only by stunting and a general chlorosis of the host plant, sometimes accompanied by a faint mottling of the foliage and slight malformation of the leaflets. Besides the common tomato, four different botanical subspecies were used, which are classified by Bailey (2) as follows: Large-leaved tomato (*L. esculentum* var. *grandifolium* B.), Cherry tomato (*L. esculentum* var. *cerasiformi* B.), Pear tomato (*L. esculentum* var. *pyriforme* B.), and Upright tomato (*L. esculentum*

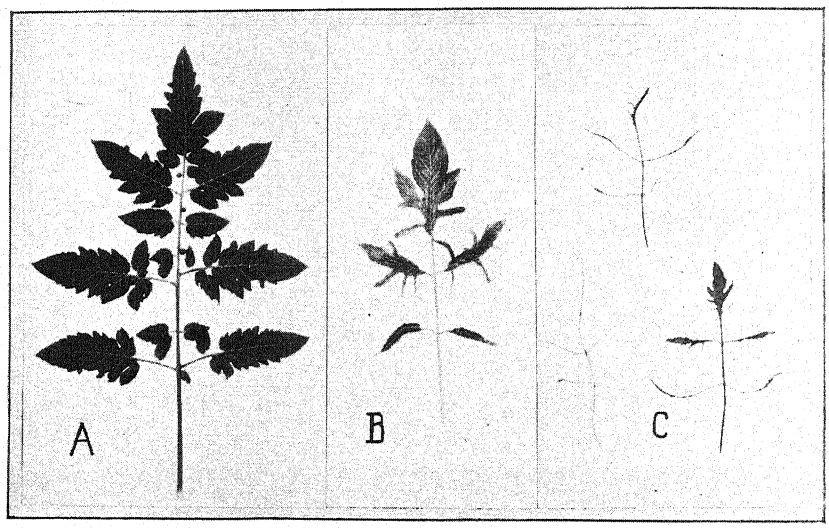


FIG. 1. A. Healthy tomato leaf from control plant. B. Tomato leaf showing approximately maximum malformation secured with the tobacco-mosaic virus; the common and characteristic symptom is mottling only. C. Tomato leaves showing fern-leaf symptoms; these are produced only sporadically with wound inoculations but may be produced at will by aphid transmission of the cucumber-mosaic virus.

var. *validum* B.). All five types of tomatoes were found to be equally susceptible to both the tobacco-mosaic virus and the cucumber-mosaic virus. Under the same environmental conditions, the symptoms produced by either virus on these varieties were essentially similar, and consequently it was assumed that the development of fern-leaf symptoms did not depend on the varietal characteristics of the host plant.

In order to facilitate the discussion of the various symptoms brought about by changes of environment, etc., the symptoms of both mosaic diseases will be described as they appear on tomato plants inoculated in the third-leaf stage and kept at a greenhouse temperature of 18–23° C.

Tobacco mosaic on tomato. The incubation period for this disease is usually about ten days at temperatures of 18–23° C. The symptom development can conveniently be divided into three successive stages, characterized, respectively, by stunting, malformation, and mottling. The early stage of the disease is marked by stunting of the whole plant and by folding or rolling of the young leaflets along their midribs. This and the slightly pendant position of the leaves give the plant a spindly appearance. In the next stage a dark green bulging appears along the veins of the leaflets together with a chlorosis of the adjoining areas. At the same time malformation of the leaflets becomes apparent as they flatten out. This malformation consists of a transformation of the normally-rounded lobes of the leaflets into delicate spine-like lobes. The laminae of the leaflets are narrower than normal, and the smaller ones may occasionally be reduced even to filiform structures. The dark green bulging is usually very conspicuous in these malformed leaves (Fig. 1, B). In the foliage appearing during the third stage, the dark green bulging and malformation are replaced by a more or less diffuse mottling. Pale green or yellow areas appear between the veins of the leaflets, while the main veins are bordered by dark green zones. In the case of the potato-leaved varieties, the young leaves are not folded in the early stage, but, on the other hand, the dark green bulging along the veins is here more conspicuous than with the other varieties.

Cucumber mosaic. In case typical fern-leaf symptoms develop, the first characteristic symptom of this disease, appearing about ten days after inoculation, is the spindly appearance of the young leaves in the terminal bud. These leaves twist around in corkscrew fashion; the young leaves, which in a normal plant start to unfold at an early stage, remain folded, curve downward, or curl up in spirals. Chlorosis of the older leaves, especially along the veins, is also an early symptom. About three weeks after inoculation, the spindly leaves have unfolded, and typical distortions are formed which result in filiform leaves (Fig. 2). These leaves, of which two or three orginate from the terminal bud, are characterized by marked reduction of the lamina, often to such an extent that only the midrib is left

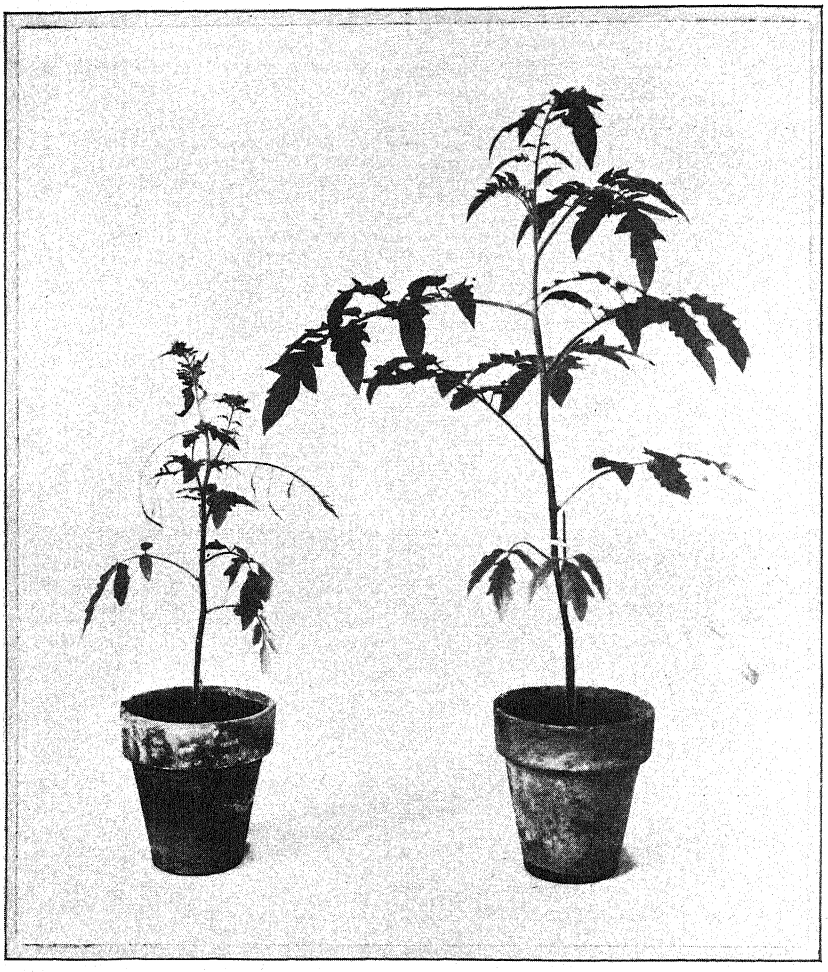


FIG. 2. Left: Tomato plant showing fern-leaf symptoms resulting from inoculation with cucumber-mosaic virus. Right: Healthy tomato plant.

(Fig. 1, C.). Besides the filiform leaves developed in the terminal bud, several others may be formed in the leaf axils. A marked rolling and folding of the leaves as well as a characteristic mottling often accompany these early symptoms. About five weeks after inoculation, when the filiform leaves are well developed, another type of symptom becomes apparent. This symptom is conspicuous by the excessive number of lateral leaflets produced. For these leaves, which may also show either mottling or general chlorosis, the name "polypinnate" leaves is suggested (Fig. 3, B, C.). No study was made of the pathological anatomy of the polypinnate leaves, but observa-

tions of intermediate stages of this condition make it appear that the excessive number of leaflets originates from an excessive branching of the vascular system of larger leaflets, while these are still in the embryonic stage. After two or three polypinnate leaves have been formed, the subsequently-developing terminal leaves usually appear more normal in their gross morphology, although mottling and chlorosis still remain. An early branching of the main stem and the prolific growth of axillary buds give the plant a bushy appearance. This and the general chlorosis and stunting are, to the casual observer, the most conspicuous symptoms.

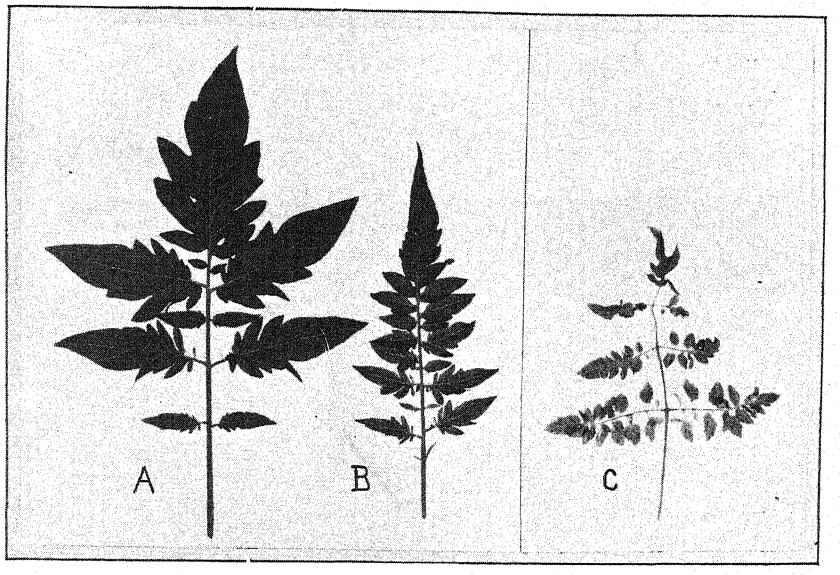


FIG. 3. A. Healthy tomato leaf from control plant. B. Polypinnate leaf from plant infected with the cucumber-mosaic virus. This abnormality is even more characteristic of infection with this virus than is the filiform leaf condition. C. The polypinnate condition, produced by the cucumber-mosaic virus, extended to the lateral leaflets.

It is usually not difficult to distinguish between the filiform leaves of a cucumber-mosaic tomato plant and the malformed leaves of a tobacco-mosaic plant of the same age. The filiform leaves show decidedly less dark green bulging or none at all, and they do not stand out so rigidly, but rather hang down flaccidly or curl around like tendrils. The mottling produced by the cucumber-mosaic virus on tomato also differs from the mottling resulting from tobacco-mosaic-virus infection. The color contrast of the adjoining areas is much less conspicuous in the case of cucumber mosaic. Instead of the larger dark and pale green areas associated with tobacco mosaic, the foliage reveals a delicate mosaic pattern of minute, sharply-defined, angular

spots of two shades of green, intensified green usually being absent. This mottling and the marked general chlorosis may often be of help to distinguish between these mosaic diseases.

THE RELATION OF ENVIRONMENTAL FACTORS TO SYMPTOM EXPRESSION ON TOMATO

From the preceding inoculation experiments it seemed more likely that the production of fern-leaf symptoms would be associated with certain environmental factors or combinations of factors, and it was, therefore, deemed desirable to study the behavior of young tomato plants inoculated with *Cucumber virus 1* under various environmental conditions in order to ascertain whether any such correlation existed. Since it had been reported by others (21) that fern-leaf symptoms could be produced by inoculation with a filtrable fraction of "tomato-mosaic" virus, which ordinarily is considered identical with *Tobacco virus 1,* a parallel series of experiments was conducted, using the latter virus for inoculum. Being considered the most probable environmental factors to affect symptom expression, the following were studied: soil temperature, atmospheric temperature, atmospheric humidity, and light intensity.

The Wiconsin constant-temperature tanks (20) were used to study the effect of soil temperature on symptom expression. The temperature of the soil was kept at approximately 15, 25, and 35° C., respectively, and the greenhouse temperature varied from 19 to 23° C. The plants were kept under observation for at least four weeks after the date of inoculation. Three series of experiments were carried out with tobacco mosaic, and essentially the same results were obtained in all cases. The effect of the soil temperature on the healthy plants was very marked. A vigorous plant was obtained at 35°, a less vigorous but well-developed plant at 25° C., while a much-stunted plant resulted at 15° C. So far as the mosaic plants were concerned, no unusual symptoms were produced. The symptoms at the three soil temperatures differed mainly in their rate of appearance and development, the most marked difference being between the plants grown at 15 and those at 25° C. A temporary partial or total masking of symptoms was obtained at soil temperatures of 25 and 35° C., which is in agreement with the findings of Johnson (15) and Dickson (9) for tobacco mosaic on tobacco. In general, it may be concluded that the results of these experiments are the same as those obtained by Johnson (15); *i.e.,* the effect of soil temperature is apparent only in so far as it affects the growth of the host plant.

The effect of soil temperature on cucumber-mosaic symptoms on tomatoes was also studied, but the results of these experiments did not permit the

drawing of definite conclusions, due to the low percentage of infection obtained.

The temperature and humidity control chambers devised by Johnson (19) were employed for the study of atmospheric relations. Three chambers with approximate temperatures of 15, 25, and 35° C., respectively, were used to study the effect of air temperature. The plants were grown in the greenhouse and removed to the chambers directly after inoculation. They were kept in the chambers for two weeks and then returned to the greenhouse for two more weeks of observation.

Besides being etiolated by the poor light conditions in the chambers at the time of the year when the tests were conducted, both healthy and diseased plants were also markedly affected by the air temperature. The plants grown at the medium temperature were the most vigorous; at 15° little growth took place and the plants were of light green color; while at 35° C. the plants were spindly with grayish green foliage.

The results of three series of chamber experiments with tobacco mosaic were of such uniformity that they may be summarized in a few statements. All inoculated plants became mosaic. At 15° C., the incubation period was 10 to 15 days; at 25 and 35° C., it was six or seven days. During the two weeks that the plants were kept in the chambers, typical symptoms developed only at the medium temperature. At the low temperature, the incubation period either exceeded two weeks or only the very early symptoms of the disease developed in the chamber. The foliage of the plants kept at 35° C. developed a dark green, sharply-defined mottling instead of dark green bulging. A week after removal from the chambers, the low- and medium-temperature plants showed typical tobacco-mosaic symptoms, while gradually these symptoms also started to appear on the new growth of the stunted and spindly, high-temperature plants.

In the case of cucumber mosaic the results were very inconsistent; the percentage of infection was variable and the incubation period exceeded two weeks, so that the effect of the temperature on the symptom expression could not be determined. In order to provide better light conditions than those prevailing in the chambers, a series of tomato plants was placed in temperature-controlled greenhouse sections directly after inoculation. The temperature fluctuations here were more marked than in the chambers, but the deviations usually did not exceed more than a few degrees Centigrade. The disadvantage of the variability in temperature was greatly compensated by the better light conditions, which permitted the exposure of the plants to the various temperatures for long periods without disturbing their photosynthetic activities. The average temperatures employed were 15, 20, 25, and 30° C., respectively. A fair amount of infection with the cucumber-mosaic virus was obtained at all temperatures and more or less typical filiform symptoms

were observed at 15, 20, and 25° C. The plants kept at 30° C. showed only semifiliform symptoms; their leaves were malformed with narrow laminae, but their transformation was not such that the term filiform was warranted.

The relation of atmospheric humidity was studied by means of the same temperature- and humidity-control chambers. The temperature of all three chambers was kept at 30° C., while relative humidities of 40, 65, and 85 per cent, respectively, were maintained. Three experiments were carried out with each mosaic disease, but in no case could any effect be detected either on the symptoms of tobacco mosaic or on those of cucumber mosaic on tomato.

In some of the experiments carried out in the chambers, necrosis was observed on the inoculated plants, which could not be attributed either to the humidity or to the temperature factor. It was noticed that this necrosis was more marked in the experiments carried out during November and December when light conditions were very poor than in the experiments performed during the succeeding months. This led to the belief that light intensity associated with mosaic disease might be the cause of the observed necrosis.

Through the kindness of Dr. W. E. Tottingham, the writer was enabled to make use of two chambers in which light as well as temperature and humidity could be controlled. Five tomato plants inoculated with tobacco mosaic were placed in each chamber directly after inoculation. Five healthy plants served as controls in each chamber. A parallel set of mosaic and healthy plants was kept in a warm greenhouse (approximately 30° C.) under natural light conditions. The temperature in the chambers was 28 to 30° C. and the relative humidity was approximately 60 per cent. The plants, which were placed on a slowly-rotating table, received their illumination only from an artificial source for eight hours each day. One chamber was provided with four 500-watt Mazda lamps, while in the other chamber only one such lamp formed the source of illumination.

After one week the mosaic plants grown under low-light-intensity conditions showed a marked necrosis of the basal leaves, while the mosaic plants receiving 2000-watt illumination showed even less necrosis than those grown under natural-light conditions in the greenhouse. Necrosis of the seed leaves on plants of this age is a natural phenomenon occurring on healthy plants even under the most favorable conditions. The necrosis of the mosaic tomatoes as caused by poor light conditions became apparent first on the basal leaves, while gradually higher leaves also were affected. In severe cases basal stem necrosis developed, causing the plant to collapse and die as if attacked by a damping-off organism. The experiment was repeated three times with similar results. With the data on hand, it is impossible to give the exact interpretation of such phenomena, but it

might be considered as additional evidence that the disturbances caused by mosaic diseases have an important bearing on the photosynthetic processes.

From the experiments with tobacco mosaic, it can be concluded that typical fern-leaf symptoms were not produced under any of the environmental conditions tried, although the symptom expression of the disease was affected in certain cases. So far as pertains to cucumber mosaic, the experiments reported here were not always satisfactory due to the variability in the percentage of infection obtained, and definite conclusions were not fully justified. Cool atmospheric temperatures seemed favorable to the development of fern-leaf symptoms; but, on the other hand, it became evident from these experiments that the variability in infection percentage with the cucumber virus was not correlated with environmental conditions.

It was then considered possible that the method of inoculation might have a more important bearing on this point and, consequently, a series of experiments was carried out to determine if any such relationship existed.

THE RELATION OF APHIDS TO THE DEVELOPMENT OF TOMATO FERN-LEAF

For a satisfactory study of the fern-leaf symptom as it results from infection with the cucumber-mosaic virus, it was, in the first place, highly desirable to be able to obtain a high percentage of infection with this virus on tomatoes. As has been shown in the previous experiments, the percentage of infection could not be correlated either with varietal susceptibility or with changes in predisposition as brought about by environmental conditions. It was then thought possible that if the virus could be provided with a more favorable substratum by bringing it in contact more directly with specific tissues, better results might be expected. In order to investigate this point, some experiments were carried out in which the inoculum was introduced into certain parts of the plants, making a special distinction between leaf parenchyma and the vascular system. The potato-leaved variety (Hudson Valley Maid) was mainly used in these experiments because of the greater simplicity of its vascular system. Insect pins, provided with very small cotton wads, were used for inoculation instead of the rather coarse dissecting needles. Where the mesophyll was the point of inoculation, special care was taken not to injure the finer veinal structures, but naturally some injury resulted. A summary of these experiments is given in table 1 and, from the data presented, it may be seen that in Experiments 1, 2, and 6, a high percentage of infection was obtained in all cases, except where the inoculation was restricted to the seed leaves. In Experiments 4 and 5 a low percentage of infection resulted in all cases but one. It thus appears that no differences in percentage of

infection were obtained, which could be attributed to the various methods of inoculation. The great variability in the percentage of infection in the various experiments cannot now be explained, although it should be noted that Experiments 1 and 2 were carried out during April when light conditions were favorable, while all other experiments were performed during November when poor light conditions prevailed.

TABLE 1.—*Results of inoculation of different parts of the plant on the percentage of infection obtained with cucumber mosaic on tomatoes*

Experiment No.	Part of plant inoculated	Plants inoculated	Plants infected	Incubation period
		Number	Number	Days
	Seed leaves	10	2	16
	Simple leaves	10	10	10
1	Seed leaves and simple leaves	10	10	10
	Seed leaves, simple leaves, and first compound leaf	10	10	10
	Control (noninoculated)	5	0	
	Mesophyll of simple leaves	10	10	9
	Veins of simple leaves	10	10	9
2	Veins of compound leaves	10	10	9
	Control (noninoculated)	5	0	
	Veins and stem	5	5	13
3	Mesophyll	5	2	13
	Stem and petioles[a]	15	4	15
	Veins	10	2	17
4	Mesophyll	10	3	17
	Control (noninoculated)	3	0	
	Leaves[b]	5	1	10
5	Stem[b]	5	2	10
	Control[b] (noninoculated)	5	0	
	Leaves[b]	15	14	9
6	Stem[b]	15	12	9
	Control[b] (noninoculated)	4	0	

[a] Inoculated by means of hypodermic needle. [b] Marglobe variety.

While this work was in progress, it was shown by Hoggan (13) that cucumber mosaic could be transmitted by the peach aphid to tomato from tobacco (*Nicotiana tabacum* L.), *N. rustica* L., and tomato. It was then but a logical step to make a comparative study of artificial inoculation by the cotton-needle method and infection by aphids. A colony of non-

viruliferous peach aphids, *Myzus persicae* Sulz., was kindly furnished by Dr. Hoggan. A supply of nonviruliferous aphids was kept in an insect-proof cage on young, healthy cabbage plants, while another supply of viruliferous aphids on young cucumber-mosaic *N. rustica* plants was kept in another cage. *Nicotiana rustica* was found to be superior to tobacco as a host plant for the aphids. All aphid-transmission experiments were carried out in the greenhouse at a temperature of 20 to 23° C., unless otherwise mentioned. Infection was brought about by transferring leaves bearing 20 or more aphids to the plants to be infected. The aphids were allowed to feed for five or six days while the plants were kept in insect-proof cages. After this period, the plants were removed to another greenhouse to be fumigated. After fumigation, the plants were kept in a cool greenhouse (20–23° C.) for observation.

A series of experiments was carried out to study the comparative effects of aphid transmission and inoculation by wounding on the symptom expression and infection percentage of cucumber mosaic on tomato. The data presented in table 2 show clearly that striking differences resulted. Where the aphid transmission method was used, 84 out of a total of 90 plants be-

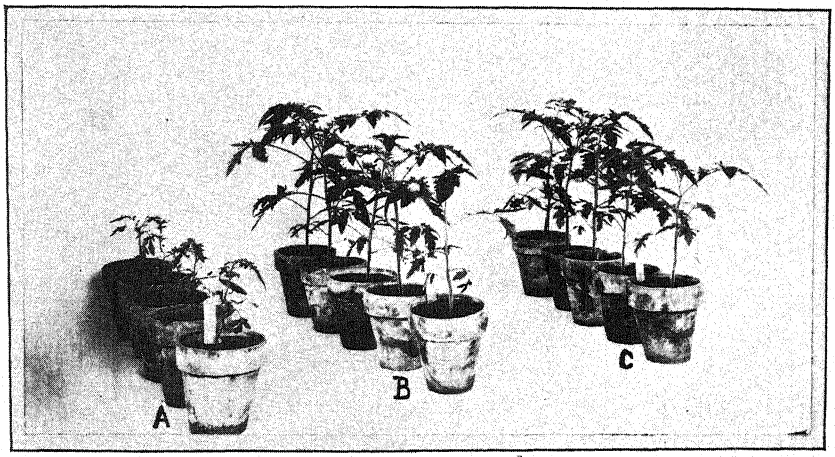


FIG. 4. A. Tomatoes infected with cucumber mosaic by means of peach aphids—B. Tomatoes inoculated with cucumber-mosaic virus by wounding (only the plant in front shows mild symptoms)—C. Control plants.

came infected, and of these, 82 showed marked fern-leaf symptoms. On the other hand, only 20 out of a total of 120 plants became infected when the plants were inoculated by the cotton-needle method and only 7 of these showed fern-leaf symptoms. (Fig. 4.) The infected plants which did not develop filiform malformations revealed their diseased condition only by

their stunted and chlorotic appearance and occasionally by mottling and slight malformations of the foliage.

TABLE 2.—*The relation of method of infection to tomato fern-leaf development*

Experiment No.	Variety	Infected by aphids			Infected by wounding			Noninoculated controls	
		Plants used	Plants infected	Plants showing fern-leaf	Plants used	Plants infected	Plants showing fern-leaf	Plants used	Plants infected
		Number	Number	Number	Number	Number	Number	Number	Number
1	Marglobe	10	10	8	5	0	0	5	0
2	Marglobe	10	9	9	10	2	1	5	0
3	Marglobe	10	10	10	45	9	1	5	0
4	Marglobe	20	18	18	20	2	2	5	0
5	Marglobe	10	9	9	10	4	0	5	0
6	Hudson Valley Maid	10	9	9	10	1	1	5	0
7	Hudson Valley Maid	10	9	9	10	2	2	5	0
8	Hudson Valley Maid	10	10	10	10	0	0	5	0
	Total	90	84	82	120	20	7	40	0

These experiments were carried out during December and January which may partly account for the extremely low percentage of infection resulting from inoculation by wounding. In the previously reported studies on environment, which were carried out under more favorable light conditions, 50 to 70 per cent of infection was often obtained, but none or very few of the infected plants developed fern-leaf symptoms. The feeding of nonviruliferous peach aphids (approximately 20 aphids per plant) on tomato plants in the third-leaf stage for two weeks produced no symptoms. Thus, positive evidence is obtained that fern-leaf symptoms on tomatoes can be produced at will by infecting young tomato plants (second- or third-leaf stage) with *Cucumber virus 1* by means of the peach aphid under the environmental conditions mentioned.

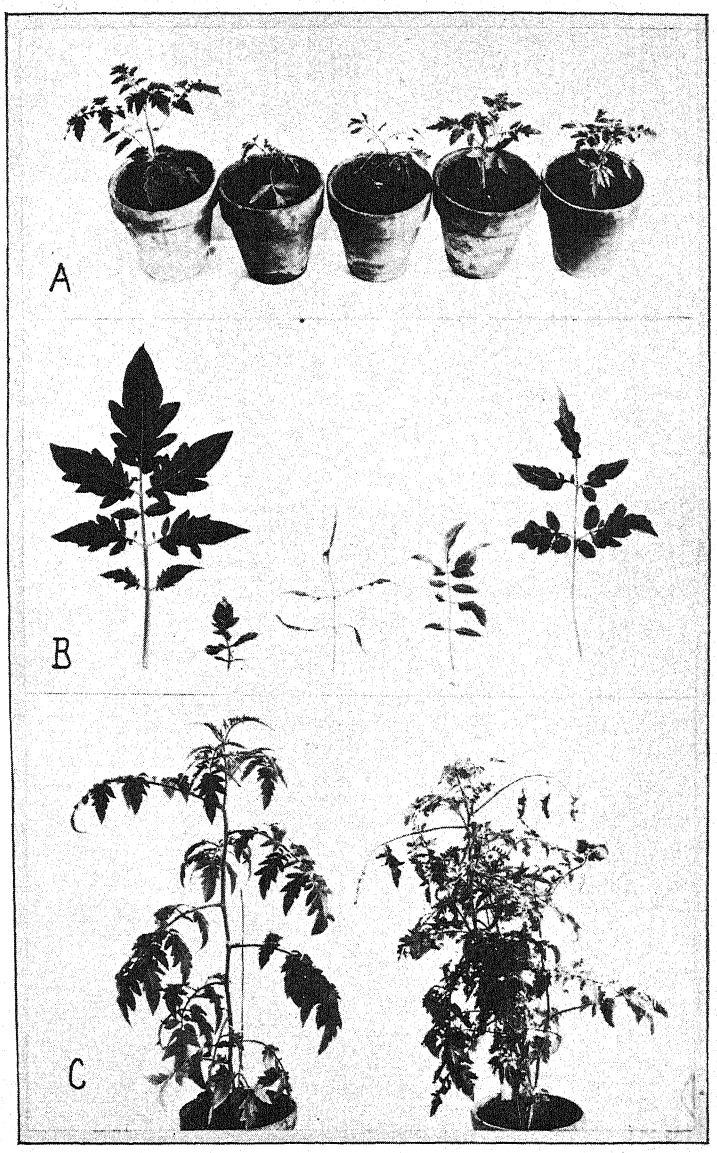


FIG. 5. A. Effect of atmospheric temperature on the development of tomato fern-leaf. Left to right: Healthy plant (grown at 20° C.); cucumber-mosaic tomato plants grown at 15, 20, 25, and 30° C., respectively. B. Leaves taken from plants shown in A. C. Left: Healthy tomato plant. Right: Cucumber—mosaic tomato plant showing early branching of main stem, prolific development of axillary buds, and filiform foliage.

If such plants infected by aphids were transferred to larger pots and allowed to grow to maturity, the following symptoms were observed: An early branching of the main stem frequently occurred and was usually followed by secondary branching. The plants were stimulated towards formation of axillary buds, which, by their prolific growth, contributed considerably to a bushy appearance of the plant. (Fig. 5, C). Mottling was always present, but a marked chlorosis and stunting were often more conspicuous symptoms. The plants blossomed, but most blossoms failed to set fruit or the fruit was shed prematurely. The leaves were malformed and often filiform; crinkling of the leaves was a common symptom. Besides crinkling, a spiral-like twisting both of the whole leaflets and of their markedly malformed lobes could be observed. More or less polypinnate leaves were found in all new growth. The polypinnate characteristic and the marked crinkling of the leaves, together with the inconspicuous mottling and prolific growth of new shoots, make cucumber mosaic on tomato relatively easy to distinguish from tobacco mosaic on the same host. In the case of tobacco mosaic neither tendril-like, filiform leaves nor polypinnate leaves are formed. Crinkling of the leaves and a marked development of new shoots are special characteristics of cucumber mosaic, while distinct mottling is usually more conspicuous on tobacco-mosaic plants. It should be borne in mind, however, that diagnosis on the basis of symptoms is very difficult, if not impossible, if both viruses manifest their symptoms simultaneously on the same host plant. In that case their symptoms will be superimposed and the presence of tobacco mosaic is difficult to detect unless inoculation experiments are carried out.

If tomato plants are infected with cucumber mosaic by means of aphids after the plants have reached a height of five inches or more, much less marked malformation is obtained and in many cases the only disease symptoms observed are stunting, chlorosis, and mottling. In the transferring of the virus to these larger plants, greater numbers of aphids were used, since the peach-aphid strain employed did not thrive well on larger plants. Two experiments, however, each involving 15 inoculated plants, gave 100 per cent of infection with cucumber mosaic but no fern-leaf.

The production of fern-leaf symptoms thus depends on the age of the plant at the time of infection, and, as will be shown later, also on the atmospheric temperature. But, even when these requirements are fulfilled, the symptoms produced are not always of the fern-leaf type if the infection has been brought about by wounding. In most cases where wounding is employed as the method of infection, the only symptoms produced are mottling and slight stunting, while in other cases chlorosis or slight malformation will result, but typical fern-leaf is produced only in relatively rare instances. There are, of course, several cases known among virus diseases where aphids

have proved to be much more efficient in the production of a high percentage of infection, but the writer knows of no case where a different type of symptoms has resulted if the infection was produced by means of aphids in comparison with artificial inoculation by wounding. Although attempts have been made to determine the cause of this behavior, an explanation for this interesting phenomenon is not now available.

THE RELATION OF ENVIRONMENTAL FACTORS TO THE DEVELOPMENT OF TOMATO FERN-LEAF PRODUCED BY APHID INFECTION

Since it was now possible to obtain a high percentage of typical fern-leaf symptoms, it was considered worth while to study once more the effect of atmospheric temperature on the symptoms produced. Forty young plants were infected by means of aphids and then subjected to different atmospheric temperatures. Five plants were placed in each of four constant-temperature chambers and five in each of four temperature-controlled greenhouse sections. Two noninoculated plants also were placed in each chamber and in each greenhouse to serve as controls. All aphid-bearing plants developed symptoms and all control plants remained healthy.

The temperatures in the greenhouse sections were 12–18° C., 18–24° C., 27–33° C., and 29–32° C., respectively. At 12–18° C. growth was very slow, but, after about four weeks, more or less filiform leaves developed on two plants, while the others showed malformation, stunting, and mottling. At 18–24° C. typical fern-leaf symptoms developed on all plants three weeks after inoculation and polypinnate leaves developed about two weeks later. At 27–33° C. the symptom expression was practically the same as at 29–32° C.; no typical fern-leaf symptoms developed on any of the plants although slight distortions and narrowing of laminae occurred. At these higher temperatures excessive branching was observed as well as the formation of polypinnate leaves, while mottling of the cucumber-mosaic type was rather conspicuous.

The temperatures of the chambers were 10–11° C., 19–21° C., 24–26° C., and 27–30° C., respectively. The disease symptoms observed in the chamber were of the same order as those described above for the greenhouse, except for partial etiolation of the plants in the chambers. No growth took place in the chambers at 10–11° C.; after six weeks the inoculated plants, which were spindly and chlorotic, showed marked leaf rolling. At 19–21° C., marked fern-leaf symptoms developed, while at the higher temperatures the disease expressed itself by excessive branching, malformation, and mottling. These experiments thus show that the optimum temperature for the formation of fern-leaf symptoms lies between 18 and 22° C.; the approximate minimum is 15° C. and the approximate maximum temperature, 25° C. (Fig. 5, A, B.)

In order to determine whether atmospheric temperature would change the predisposition of the host, ten young tomato plants grown in a warm greenhouse (30° C.) and ten others grown in a cool greenhouse (20° C.) were infected at the same time by means of aphids. After fumigation, both groups were kept in the cool house. No difference was noticed either in the incubation period or in the percentage of infection produced. Typical, stunted, filiform plants resulted in both cases.

The effect of light intensity on cucumber-mosaic tomatoes was now also studied by using aphids for transmission of the virus. The experiment was performed in the same way as previously described for tobacco mosaic, except that the temperature of the chambers was now kept at 20 to 23° C. with a relative humidity of 45 to 50 per cent. All the infected plants in the high-light-intensity chamber developed typical filiform symptoms, while those kept under poor light conditions showed severe symptoms of wilting and necrosis which resulted in the death of some of the plants.

DISCUSSION

The experimental results reported in this paper may contribute to the explanation of certain contradictory results obtained by previous investigators and may clear up some of the existing confusion concerning tomato mosaic. It is of interest, therefore, to consider once more the statements found in the literature in the light of the experimental results obtained by the writer.

Westerdijk's (30) failure to cross-inoculate the mosaic diseases of tobacco and tomato which she was studying may be explained by the fact that cucumber-mosaic tomato plants served as sources for inoculum. The artificial inoculation with cucumber-mosaic virus is much more difficult than with tobacco-mosaic virus, especially when larger plants are used, as was often the case in Miss Westerdijk's work. Moreover, the cucumber-mosaic virus soon loses its virulence after extraction, which is another factor that might account for her negative results. From the illustrations in her paper, it seems very evident that she did observe both virus diseases. Figure 1 and figure 3 of her paper show mottling and malformation, very typical for tobacco mosaic; while marked filiform symptoms are reproduced in figure 5. The latter may have resulted from aphid infection.

In describing the symptoms of tomato mosaic, Gardner and Kendrick (12) state that "the leaflets may be distorted and crumpled or reduced to narrow ribbons. Severely diseased plants may be greatly stunted and bushy with a yellowish cast and the leaves so reduced as to warrant the term 'fern-leaf.' Such plants bear only stunted fruits or none at all." With these words they present an extact picture of a cucumber-mosaic

tomato plant, and this would indicate that they, also, were dealing, in part at least, with cucumber mosaic.

Lesley and Lesley (23) have reported the occurrence of "wiry" tomato plants resembling in appearance tomatoes affected with fern-leaf mosaic but differing from these by the hereditary nature of the wiry character. The results of their crossing experiments cannot be accepted as positive evidence, however, since insect control was not provided. The fact that the wiry symptoms were not observed to be transmitted in the field is the only evidence presented to establish its noninfectious character.

With the facts at hand, special attention should be given to the work of Kraybill and Eckerson (21). According to these investigators, the juice of tomatoes affected with tomato mosaic can, by means of filtration, be separated into two fractions, one producing mottling symptoms only, while the other causes the formation of fern-leaf symptoms. In regard to their work it should be pointed out in the first place that, judging by their illustrations, their conception of fern-leaf differs from the commonly accepted idea of filiformity. The term fern-leaf is applied by them even to slightly narrowed and malformed laminae, such as frequently occur on tomatoes affected with tobacco mosaic. Only the plant pictured in the center of figure 4 of their paper (21) shows symptoms which suggest the presence of typical filiformity. A careful reading of their paper further reveals the fact that most, if not all, of their inoculations were carried out with inoculum that had been stored for more than one week. Such an aging process will naturally exclude the presence of any cucumber-mosaic virus that might have been present originally. In a more recent publication by Kraybill et al. (22), it is claimed that this filtrate retains its qualities even after being heated for two and a half hours at 126° C. Such a treatment will exclude the presence of cucumber-mosaic virus as well as tobacco-mosaic virus in the inoculum. With the meager data at hand, it is at the present time impossible to form an opinion on this matter which seems to add an entirely new angle to the plant-virus-disease problem.

Of the various types of tomato-mosaic symptoms recognized by Bewley (4) the "tendril-type" is very likely a manifestation of cucumber mosaic. It would be difficult to make any definite statement concerning the other types, especially where illustrations are lacking. It might be mentioned, however, that two types of mosaic on cucumber occur in England, as stated by the same writer. According to the descriptions given by Weber and Ramsey (29), cucumber mosaic on tomatoes may be a disease of importance in Florida. Of the three types distinguished by them, it would seem that the linear type and possibly also the crinkle type are manifestations of cucumber mosaic, while the mottle type resembles tobacco mosaic.

Although not having been able to make a field survey, the writer is inclined to believe that cucumber mosaic on tomatoes is of more frequent occurrence than might be expected at first thought. The occasional appearance of fern-leaf or filiform symptoms cannot be considered as a measure of the distribution of this disease. As has been pointed out in the experimental part of this paper, marked filiformity will result only if the plant is infected at a very early age. Considering the fact that the plants usually are sown in hotbeds, aphid infestations would hardly be expected in the seed bed, and, especially in temperate climates, mass infection thus becomes improbable. The plants used for field planting have often grown beyond the stage where filiform symptoms would result from aphid infection in the field, and the symptoms produced are of a different type and are easily mistaken for tobacco mosaic. Another important factor to be considered in relation to field infection is the migration of the peach aphid. It is possible that where the tomato serves as a secondary host for the peach aphid, the plants will have reached such a size at the time of migration that typical fern-leaf symptoms can no longer be expected. Since other species of aphids have not been studied in connection with the fern-leaf disease, they can at present not be included in the discussion. The more frequent occurrence of fern-leaf under greenhouse conditions can be explained by the increased chance for aphid infestations.

SUMMARY

1. Fern-leaf of tomatoes is chiefly characterized by the presence of filiform leaflets. This symptom has previously been shown to result from virus infection, but its comparatively rare and sporadic natural occurrence, together with the difficulty of reproducing it at will, prompted further investigation.

2. Typical fern-leaf symptoms could not be produced with the ordinary tobacco- or tomato-mosaic virus (*Tobacco virus 1*) under any of the environmental conditions to which the infected host was submitted.

3. Fern-leaf may occasionally be produced by artificial infection of the tomato with the cucumber-mosaic virus (*Cucumber virus 1*), as previously reported by Johnson.

4. Tomato fern-leaf can be produced regularly and at will by infecting young tomato plants with the cucumber-mosaic virus by means of the peach aphid (*Myzus persicae*).

5. The optimum atmospheric temperature for the expression of fern-leaf symptoms lies between 18 and 22° C.; the approximate minimum temperature is 15° C. and the approximate maximum is 25° C.

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EFFECT OF SEED-POTATO TREATMENT ON YIELD AND RHIZOCTONIA IN NORTHEASTERN MAINE FROM 1925 TO 1928[1] [2]

E. S. Schultz,[3] L. O. Gratz,[4] and Reiner Bonde[5]

Introdution

Tests of seed-potato treatments conducted in many potato-growing localities by others have shown that corrosive sublimate is one of the most effective treatments for controlling Rhizoctonia.[6] Although the old standard treatments controlled certain tuber diseases, they frequently failed to increase the yield. In fact, treating occasionally induced significant decreases in yield. Moreover, the additional time and labor involved in treating potatoes greatly contributed to the failure of the majority of the potato growers to treat seed potatoes. This situation encouraged additional investigations on the improvement of seed-treatment materials and methods.

The introduction of hot formaldehyde by Melhus[7] is a distinct improvement in some ways over the older methods. This method greatly reduces the total period of time of treating (though not necessarily the actual labor time per bushel) and is fully as effective as the former standard methods.

The organic mercuries recently developed by the Bayer and du Pont companies[8] have reduced the time of treatment to a minimum and have provided for the treatment of cut as well as whole seed. The yields secured by the use of these newer compounds have compared favorably with those obtained with the old standard treatments. In general, however, they have been reported as not controlling Rhizoctonia as well as corrosive sublimate.

[1] Conducted as a cooperative project between the Bureau of Plant Industry, U. S. Department of Agriculture, the Florida Agricultural Experiment Station, and the Maine Agricultural Experiment Station.

[2] The authors wish to express their gratitude to Dr. W. J. Youden, of the Boyce Thompson Institute for Plant Research, Yonkers, N. Y., for helpful suggestions and criticisms on plans for replicated plots, and to the Bayer and du Pont companies for furnishing various organic-mercury compounds and treatment formulae for their products.

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[4] Associate Plant Pathologist, Florida Agricultural Experiment Station.

[5] Associate Plant Pathologist, Maine Agricultural Experiment Station.

[6] *Rhizoctonia solani* Kühn, imperfect form of *Corticium vagum* B. & C.

[7] Melhus, I. E. Seed treatment with hot solutions of formaldehyde and mercuric chlorid. Phytopath. 8: 81. 1918.

[8] Now consolidated as the Bayer-Semesan Company, Inc., of New York.

Experience has shown that conflicting results may be obtained from the same treatment in different localities in any one year or in different seasons in any one region.

In view of (1) the new materials and methods, (2) the variation in results according to locality, (3) the importance of Aroostook County (northeastern Maine) as a potato region, and (4) the absence of recent tests of new or standard treatments in Aroostook County, it was thought desirable to conduct such tests in this particular area. This was done from 1925 to 1928, inclusive. A further reason for this work was the hope of determining some of the causes of unexplained variation, especially with reference to controlling Rhizoctonia and increasing the yield.

GENERAL EXPERIMENTAL METHODS

Irish Cobbler tubers uniformly covered with sclerotia of Rhizoctonia were used in the experiments. Clean tubers also were treated with corrosive sublimate to obtain information on soil infestation by Rhizoctonia. In 1925 and 1926 the clean tubers represented a different strain of Irish Cobblers from the diseased lots so that no comparison of yield between the two lots was made in those seasons. In 1927 and 1928, however, clean and diseased tubers were selected from the same strain, thus providing for comparison of yield between the two lots.

The same workman cut the tubers into 1.5-ounce seed pieces for all four seasons, thus providing as uniform seed as possible under representative field conditions. The seed pieces were planted by hand at 14-inch intervals in rows 3 feet apart. Commercial fertilizer, used at the rate of 1500 pounds per acre, was applied in the rows with a planter so that the soil and fertilizer were completely mixed before planting.

During each of the four seasons, the plots were located on apparently uniform Caribou loam on Aroostook Farm, Presque Isle, Maine, where a 3-year rotation with oats, clover, and potatoes is followed.

The potatoes were treated according to the formulae recorded in the tables and were stored in bushel crates in a cool cellar for about two weeks before planting. The tubers treated with hot formaldehyde were covered with burlap for one hour immediately after treating.

Observations were made on stand, relative vigor, seed-piece decay, stem lesions, tuber infection, and yield. Readings on seed-piece decay were taken only in 1927 and 1928. The observations on seed-piece decay and stem lesions were made when the plants were about 4 to 6 inches above the soil; stems showing only slight Rhizoctonia lesions were included in the Rhizoctonia percentages. The percentages of seed-piece decay include partly as well as completely decayed seed pieces. The percentage of tuber infection includes any tubers showing Rhizoctonia sclerotia regardless of

the extent of the infection. This percentage was obtained from representative tuber samples of each replication. In order to obtain reliable data on tuber infection it was necessary to wash the tubers each year at harvest, which began as soon as the foliage had matured.

The yields secured in the 1925 and 1926 experiments were calculated on the basis of total yields, while those obtained in 1927 and 1928 were based on the weights of marketable tubers. Tubers were graded according to the standards of the United States Department of Agriculture. The percentage differences in yield were obtained by comparing the yield of each treated lot with that of the corresponding untreated diseased control. It was found that the percentage differences in yield of marketable tubers, primes, or total tubers were similar.

The formulae $\text{PEM} = \frac{.6745 \times \text{standard deviation}}{\sqrt{\text{no. of items}}}$ and $\text{PED} = \sqrt{a^2 + b^2}$, where a = probable error of the mean of the treated lot and b = probable error of the check with which the treated lot is compared, were used for determining the significance of the differences in yield. Odds as listed by Pearl and Miner[9] were used.

EXPERIMENTAL DATA FOR 1925

Procedure. Twelve treatments as recorded in table 1 were conducted. Each treated diseased lot was planted beside an untreated diseased control and replicated in four 200-foot rows. Every fourth row represented a treated healthy control as an index on soil infestation. The seed pieces were dropped in the open rows and covered immediately after planting. Observations on stand, stem lesions, tuber infection, and yield are recorded in table 1.

Stem lesions. The sprouts from 25 hills per replication, or 100 hills of each lot, were examined for Rhizoctonia lesions to determine their correlation with soil infestation and the disease on the seed pieces and tubers. Clean and infected stems frequently appeared in the same hill where the extent of infection varied from slight lesions to girdling of the shoots. As indicated in table 1, one to 39 per cent of the hills in the treated diseased lots manifested stem lesions, while 65 per cent of the hills in the nontreated controls showed stem infection. Only one per cent of the treated healthy lots showed lesions. This indicates that the percentage of stem lesions was generally correlated with the amount of seed-piece infection and with the control of their infection by treatment.

The lots treated with hot and cold corrosive sublimate showed only 1 per cent each of stem infection and indicated the best control. From the

[9] Pearl, Raymond, and John Rice Miner. A table for estimating the probable significance of statistical constants. Maine Agr. Exp. Sta. Bul. 226: 85–88. 1914.

TABLE 1.—*Results obtained with seed-potato treatments in northeastern Maine in 1925*

Treatment[1]	Percentage of stand	Percentage with stem lesions	Percentage with tuber infection	Calculated yield in bushels per acre		Increase per acre by treating		Odds[2]
				Treated	Not treated	Calculated bushels	Percentage	
Corrosive sublimate 1–3000 and .1% HCl 1½ hr.	97.7	19	10.3	246.8 ± 2.5	223.5 ± 4.5	23.3 ± 5.1	10.4 ± 2.3	519:1
Uspulun 1–400 1 hr.	98.4	22	16.1	274.0 ± 6.6	249.9 ± 4.4	24.1 ± 8.0	9.7 ± 3.2	22:1
Uspulun 1–400 50° C. 2 min.	98.8	18	11.9	305.2 ± 5.8	281.5 ± 9.6	23.7 ± 11.2	8.4 ± 4.0	5:1
Semesan 1–400 1 hr.	98.4	20	11.0	242.5 ± 4.5	223.5 ± 4.5	19.0 ± 6.4	8.5 ± 2.8	22:1
Bayer-compound 1–400 1 hr.	99.5	39	26.0	268.3 ± 7.7	249.9 ± 4.4	18.4 ± 8.9	7.4 ± 3.6	9:1
Bayer-compound 1–400 50° C. 2 min.	99.4	28	11.0	299.4 ± 9.4	281.5 ± 9.6	17.9 ± 13.4	6.4 ± 4.8	2:1
Corrosive sublimate 1–1000 1½ hr.	99.1	1	3.8	278.6 ± 2.6	270.1 ± 3.7	8.5 ± 4.5	3.1 ± 1.7	4:1
Corrosive sublimate 1–500 50° C. 2 min.	98.6	1	2.3	275.5 ± 3.9	270.1 ± 3.7	5.4 ± 5.3	2.0 ± 2.0	1:1
Semesan 13U dust 3 oz. per bu.	99.4	8	7.6	286.4 ± 5.4	282.2 ± 5.8	4.2 ± 8.0	1.5 ± 2.8	0
Formaldehyde 1–120 50° C. 2 min.	97.2	14	6.4	296.7 ± 1.3	294.9 ± 2.6	1.8 ± 2.9	0.6 ± 1.0	0
Bayer dust (tubers presprinkled) 4 oz. per bu.	98.6	7	7.8	283.1 ± 4.0	282.2 ± 5.8	0.9 ± 7.1	0.3 ± 2.5	0
Formaldehyde 1–240 2 hrs.	97.5	15	6.3	287.1 ± 2.4	294.9 ± 2.6	−7.8 ± 3.5	−2.6 ± 1.2	6:1
Check, diseased, not treated	96.4	65	22.3					
Check, clean, corrosive sublimate 1–1000 1½ hr.	96.0	1	2.0					

[1] Whole tubers treated in all lots.

[2] Significance is shown by odds of over 22 to 1.

percentage of stem infection, especially in the untreated lots, one might have expected considerable unevenness in stand later. However, at blossoming time the general appearance of the tops in the treated and untreated lots was the same.

Tuber infection. On September 17, shortly after the death of the tops, the plots were harvested and one-bushel samples per replication, selected from representative sections of the plot, were reserved for observations on tuber infection. Each sample of approximately 200 tubers was examined for Rhizoctonia sclerotia.

The data on tuber infection recorded in table 1 disclose the fact that corrosive sublimate gave the best control. With the exception of the Bayer-compound-treated lot, showing slightly more tuber infection than the diseased control, the treated lots manifested less infection than the diseased control. A close correlation was found between stem infection and tuber infection. In general, smoother, cleaner, and less deformed tubers appeared in the treated than in the nontreated lots, although the relative percentage of badly infected tubers was so small that the appearance of the entire stock was not marred.

Yield. Significant increases in yield were obtained from the lots treated with acidulated corrosive sublimate, Uspulun and Semesan. With the exception of the lot treated with cold formaldehyde, which gave a slight decrease in yield, the other treated lots showed slight increases in yield over the diseased control. It is interesting to note that the highest increases in yield were obtained from the treated lots possessing relatively high percentages of stem and tuber infection, which suggests that Rhizoctonia infection was not the only determining factor but that possibly injurious effects from certain treatments may have reduced yield more than did the effects from Rhizoctonia.

EXPERIMENTAL DATA FOR 1926

Procedure. As recorded in table 2, eighteen treatments were compared in 1926. Three replications of 25 hills each were conducted for each treatment. Every fifth, sixth, and seventh row represented a clean nontreated, a clean treated, and a diseased nontreated control, respectively.

In view of the small number of replications, individual hill weights of all the lots were taken to secure adequate comparisons between the diseased treated lots and the nontreated controls. Since the clean treated and nontreated lots represented a different strain of Irish Cobblers from the diseased lots, comparisons of yield between them were not made.

Formulae and results of the treatments are recorded in table 2.

Stem lesions. As shown in table 2, all treatments reduced the number of stem lesions. The treated diseased lots manifested 0 to 33 per cent of

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TABLE 2.—*Results obtained with seed-potato treatments in northeastern Maine in 1926*

Treatment	Tubers when treated[1]	Percentage of stand	Relative vigor	Percentage with stem lesions	Percentage with tuber infection	Calculated yield in bushels per acre		Increase per acre by treating		
						Treated	Not treated	Calculated bushels	Percentage	Odds[2]
Semesan Bel 1–20 dip	C	96.0	91.7	1.7	2.0	248.3 ± 7.8	205.4 ± 7.8	42.9 ± 11.0	20.9 ± 5.4	116:1
Corrosive sublimate 1–500 52° C. 2 min.	W	96.0	87.3	5.0	3.0	261.3 ± 9.1	218.4 ± 7.8	42.9 ± 12.0	19.6 ± 5.5	64:1
12 Bel 1–10 dip	C	90.7	86.7	0.0	7.4	241.8 ± 9.1	205.4 ± 7.8	36.4 ± 12.0	17.7 ± 5.8	22:1
Formaldehyde 1–120 52° C. 2 min.	W	96.0	83.3	13.3	12.6	240.5 ± 9.1	205.4 ± 7.8	35.1 ± 12.0	17.1 ± 5.8	19:1
Semesan Bel dust 3 oz. per bu.	C	97.3	91.7	20.0	29.3	236.6 ± 6.5	205.4 ± 7.8	31.2 ± 10.1	15.2 ± 4.9	26:1
Semesan Bel 1–10 dip	W	100.0	88.6	33.3	47.3	230.1 ± 5.2	198.9 ± 6.5	31.2 ± 8.3	15.7 ± 4.2	95:1
Bayer Dust II–6 3 oz. per bu.	C	100.0	95.7	15.0	27.0	226.2 ± 5.2	198.9 ± 6.5	27.3 ± 8.3	13.7 ± 4.2	37:1
Uspulun 1–400 1 hr.	C	94.7	90.3	20.0	5.2	224.9 ± 7.8	198.9 ± 6.5	26.0 ± 10.1	13.1 ± 5.1	11:1
Formaldehyde 1–240 2 hr.	W	100.0	94.0	10.0	10.0	247.0 ± 5.2	218.4 ± 7.8	28.6 ± 9.4	13.1 ± 4.3	26:1
Semesan 1–400 1 hr.	C	98.7	82.7	13.3	13.4	244.4 ± 6.5	223.6 ± 9.1	20.8 ± 11.2	9.3 ± 5.0	4:1
Bayer-compound 1–400 1 hr.	C	89.3	88.3	15.0	22.6	214.5 ± 7.8	198.9 ± 6.5	15.6 ± 10.1	7.8 ± 5.1	2:1
37 Bel dust 3 oz. per bu.	C	97.3	85.0	3.3	10.3	236.6 ± 6.5	223.6 ± 9.1	13.0 ± 11.2	5.8 ± 5.0	1:1
12 Bel dust 3 oz. per bu.	C	98.7	92.3	13.3	24.3	244.4 ± 5.2	232.8 ± 9.1	11.6 ± 10.5	5.0 ± 4.5	1:1
Bayer Special 90 1–20 dip	C	98.7	92.7	1.7	14.6	247.0 ± 6.5	241.8 ± 6.5	5.2 ± 9.2	2.2 ± 3.8	0
Bayer Special 89 1–20 dip	C	94.7	95.0	23.3	27.9	235.3 ± 6.5	241.8 ± 6.5	−6.5 ± 9.2	−2.7 ± 3.8	0
Corrosive sublimate 1–1000 1½ hr.	W	84.0	78.7	5.0	2.0	210.6 ± 9.1	218.4 ± 7.8	−7.8 ± 12.0	−3.6 ± 5.5	0
37 Bel 1–10 dip	C	88.0	71.0	1.6	0.0	211.9 ± 9.1	223.6 ± 9.1	−11.7 ± 12.8	−5.2 ± 5.7	1:1
Semesan Bel dust 3 oz. per bu.	W	97.3	90.0	25.0	36.9	222.3 ± 6.5	241.8 ± 6.5	−19.5 ± 9.2	−8.1 ± 3.8	5:1
Check, diseased, not treated		96.3	79.9	49.3	39.5	See next column				
Check, clean, not treated		98.7	97.5	1.0	4.9					
Check, clean, corrosive sublimate 1–1000 1½ hr.	W	99.0	96.8	1.3	4.0					

[1] C = cut seed pieces treated; W = whole tubers treated.
[2] Significance is shown by odds of over 22 to 1.

stem infection, while the diseased control showed 49 per cent and the treated clean but one per cent of infection, thus indicating a close association of seed-piece infection with stem lesions.

The most effective control was found in the lots treated with 12 Bel, 37 Bel, Semesan Bel 1–20, Bayer Special 90, 37 Bel dust, and cold and hot corrosive sublimate showing 0, 1.6, 1.7, 1.7, 3.3, 5, and 5 per cent of stem infection, respectively. Different concentrations and forms of the same products gave inconsistent results; the 37 Bel dip and dust treatments were about equally effective in controlling stem infection, while Semesan Bel dip 1–20 on cut seed appeared much more effective than Semesan Bel dip 1–10 on whole tubers or dust on cut seed. The results indicate that the liquid treatments controlled stem lesions better than the dusts.

Tuber infection. As in 1925, observations on tuber infection disclosed a close correlation between stem lesions and tuber infection. 37 Bel dip gave perfect control, while the lots treated with cold corrosive sublimate and Semesan Bel dip 1–20 gave 2 per cent of infected tubers. The non-treated clean and diseased controls produced 4.9 and 39.5 per cent of tuber infection, respectively. The lot treated with Semesan Bel dip 1–10 manifested 47.3 per cent of infected tubers and was the only treated lot which showed more disease than the diseased control. The extent of Rhizoctonia infection on the tubers was similar to that in 1925 and did not affect materially the appearance of the entire stock.

Yield. Significant increases in yield were obtained from the lots treated with Semesan Bel dip on whole and cut tubers, 12 Bel dip, Bayer Dust 11–6, Semesan Bel dust on cut tubers, hot corrosive sublimate, and hot and cold formaldehyde. The lots treated with cold corrosive sublimate, 37 Bel dip, Semesan Bel dust, and Bayer Special 89 dip yielded slightly less than the diseased control. It is noteworthy that, as in 1925, certain treatments, giving very good control of Rhizoctonia, did not increase the yield. This suggests that possible injury from treatment may be more detrimental to the plants than Rhizoctonia. The hot corrosive-sublimate lot produced next to the highest increase in yield, while the one treated with cold corrosive sublimate slightly underyielded the control, indicating that the same material differently applied may vary greatly in its effects in the same season. Cold formaldehyde effected a significant increase in yield in 1926, while in 1925 this treatment produced a slight decrease in yield, showing that the effects of the same material may vary widely in different seasons in the same locality. That low vigor is not invariably associated with low yield is shown by the high vigor but low yields from the lot treated with Bayer Special 89 dip.

Although the yields of the healthy lots were not compared with the diseased lots on account of difference in origin of the two, it is noteworthy

that the diseased lot treated with the cold corrosive sublimate manifested a relative vigor of 78.7, while the clean lot given the same treatment showed a relative vigor of 96.8. This indicates that a different reaction may be obtained from different lots of the same variety of seed potatoes treated with the same treatment in the same season and locality.

EXPERIMENTAL DATA FOR 1927

Procedure. The clean and diseased tubers were obtained from the same commercial stock of Irish Cobblers. The treated lots and nontreated controls were planted in plots arranged according to the chessboard plan (Table 5), a modification of the magic-square system. This plan provides for better distribution of the different lots from the standpoint of competition and soil variations than do the systems followed in 1925 and 1926. Moreover this plan provides for more satisfactory biometrical measurements with more replications, but with fewer plants per replication, than does that of 1925. Although ten 25-hill plots per treatment were made, yields were calculated for nine replications in view of considerable soil variation in one replication.

Observations on stem lesions and seed-piece decay were made on 52 hills per treatment which were planted in a plot adjoining the one for yields and were examined when the plants were about 4 to 6 inches above the soil.

The formulae and results of the treatments are recorded in table 3.

Seed-piece decay. The treatments apparently inhibited seed-piece decay. The nontreated diseased control showed decay in 100 per cent of the seed pieces in contrast with four of the treated diseased lots which remained free of decay. The nontreated clean control developed 33 per cent of decay, while the corrosive-sublimate-treated lot showed none. However, the clean lot treated with 2B dip had 15 per cent more decay than the nontreated clean control, thus suggesting that this might have caused seed-piece injury and subsequent decay. The diseased nontreated control, showing 66 per cent more decay than the clean nontreated control, seems to indicate that Rhizoctonia on the seed pieces favors seed-piece decay. However, further work on this phase of seed-potato treatment is necessary before all factors influencing seed-piece decay are known. Contrary to expectations, seed-piece decay was controlled as well by treating whole tubers as by treating cut tubers.

Stem lesions. Two clean treated lots and the clean nontreated control did not develop stem infection, while the diseased treated lots developed from 2 to 40 per cent of stem lesions. Corrosive sublimate and cold formaldehyde gave the best control, with lesions on but 2 per cent of the plants. It is interesting to note that the diseased check with 8 per cent

TABLE 3.—*Results obtained with seed-potato treatments in northeastern Maine in 1927*

Treatment	Tubers when treated[1]	Percentage stand	Relative vigor	Percentage with seed-piece decay	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers			
							Yield per acre in bushels	Increase in yield over diseased untreated check		
								Bushels per acre	Percentage	Odds[2]
Corrosive sublimate 1–1000 1½ hr.	W	98.8	88.5	0.0	2.0	14	418.3 ± 5.9	78.5 ± 8.1	23.1 ± 2.4	M[3]: 1
Formaldehyde 1–240 2 hr.	W	96.4	82.4	0.0	2.0	26	411.5 ± 3.9	71.7 ± 6.7	21.1 ± 2.0	M: 1
Formaldehyde 1–120 52° C. 3 min.	W	93.2	81.6	0.0	26.0	25	403.9 ± 6.8	64.1 ± 8.8	18.9 ± 2.6	434782: 1
Semesan Bel 1–10 dip	W	97.2	86.6	24.0	20.0	33	399.3 ± 8.4	59.5 ± 10.0	17.5 ± 2.9	19230: 1
Semesan Bel 1–10 dip	C	96.8	87.2	27.0	40.0	43	410.0 ± 4.3	70.2 ± 7.0	20.7 ± 2.1	M: 1
2 B 1–20 dip	C	94.0	81.8	24.0	16.0	42	377.4 ± 4.8	37.6 ± 7.3	11.1 ± 2.2	1350: 1
2 B 1–30 dip	C	91.6	76.4	62.0	22.0	39	356.7 ± 6.1	16.9 ± 8.3	5.0 ± 2.4	4: 1
12 Bel 1–20 dip	C	98.8	92.0	2.0	36.0	38	398.8 ± 4.0	59.0 ± 6.8	17.4 ± 2.0	M: 1
21 Bel 1–20 dip	C	98.4	88.0	2.0	12.0	34	394.2 ± 4.6	54.4 ± 7.2	16.0 ± 2.1	M: 1
37 Bel 1–20 dip	C	98.8	87.0	0.0	34.0	41	402.9 ± 5.2	63.1 ± 7.6	18.6 ± 2.2	M: 1
62 B 1–20 dip	C	99.6	89.5	0.0	17.0	30	402.7 ± 3.7	62.9 ± 6.6	18.5 ± 1.9	M: 1
Dipdust 1–20 dip	C	97.6	91.4	14.0	22.0	41	407.0 ± 5.1	67.2 ± 7.5	19.8 ± 2.2	M: 1
Dipdust 1–20 dip	W	98.0	89.6	2.0	29.0	36	402.4 ± 9.4	62.6 ± 10.9	18.4 ± 3.2	19230: 1
Bayer Special 181 1–40 dip	C	98.0	88.7	14.0	32.0	35	393.6 ± 6.5	53.8 ± 8.5	15.9 ± 2.5	19230: 1
Bayer Special 181 1–40 dip	W	99.2	86.4	36.0	36.0	37	386.6 ± 5.5	46.8 ± 7.8	13.8 ± 2.3	19230: 1
Diseased, check, untreated		89.2	71.8	100.0	8.0	27	339.8 ± 5.5			
Clean, check, untreated		98.4	89.0	33.0	0.0	18	398.9 ± 6.1	59.1 ± 8.2	17.4 ± 2.4	434782: 1
Clean, corrosive sublimate 1–1000 1½ hr.	W	98.4	85.4	0.0	0.0	15	415.7 ± 4.9	75.9 ± 7.4	22.3 ± 2.2	M: 1
Clean 2 B 1–20 dip	C	97.6	86.0	48.0	0.0	21	389.2 ± 5.3	49.4 ± 7.6	14.5 ± 2.2	19230: 1
Clean, Dipdust 1–20 dip	C	99.2	93.7	6.0	10.0	15	415.3 ± 6.1	75.5 ± 8.2	22.2 ± 2.4	M: 1

[1] W = whole tubers treated; C = tubers treated after cutting.
[2] Significance is shown by odds of over 22 to 1.
[3] M = a million or more.

stem lesions showed considerably less infection than most of the treated diseased lots, which probably indicates that the extensive seed-piece decay in this lot might have inhibited Rhizoctonia from infecting the stems. Only a trace of Rhizoctonia hills with aerial tubers and of black-leg plants appeared in the plots.

Tuber infection. The percentage of infected tubers in the different treated diseased lots ranged from 14 to 43. Corrosive sublimate gave the best control. It is noteworthy that the diseased control, with only 27 per cent tuber infection, showed less Rhizoctonia on the tubers than most of the treated lots. This was contrary to the results of tuber infection obtained from the diseased control in 1925 and 1926, when, with one exception, it gave the highest percentage of infected tubers. Whether the extensive seed-piece decay in the diseased control or variations in soil infestation, as suggested by relatively high tuber infection in the clean controls, influenced the results was not determined. With a few exceptions, probably induced by variations in soil infestation, a rather close correlation appears between stem lesions and tuber infection.

Yield. As recorded in table 3, significant increases in yield were obtained with all treatments except duPont 2B dip 1–30 which effected only a slight increase over the diseased control. It is interesting to note that the yield from the corrosive-sublimate treatment was from 1 to 18 per cent greater than that from other treatments. However, only slightly lower yields resulted from treatments with Semesan Bel and Dipdust on cut seed and with cold formaldehyde than from corrosive sublimate. DuPont 2B apparently was injurious.

The greater number of treated lots showing increases in yield than in 1925 and 1926 may be due to more extensive seed-piece decay as well as more Rhizoctonia infection in 1927 than during the two previous seasons. These results suggest that the control of seed-piece decay by treating was one of the primary factors contributing towards significant increases in yield. A relative vigor below 80 was apparently associated with the lowest yields, but the highest relative vigor was not correlated with the highest yields.

EXPERIMENTAL DATA FOR 1928

Procedure. A similar series of treatments with the same plot arrangement as in 1927 was conducted in 1928. The Bayer products, Dipdust and Bayer Special 181, were the same as those used in 1927, so that the results of these materials and of the old standard treatments can be compared for the two seasons. Certain modifications of the du Pont products, Semesan Bel and 76 Bel, prevent similar comparisons with them. Ten 26-hill replications per treatment were made. The formulae and results of the treatments are recorded in table 4.

TABLE 4.—*Results obtained with seed-potato treatments in northeastern Maine in 1928*

Treatment	Tubers when treated[1]	Percentage of stand	Relative vigor	Percentage with seed piece decay	Percentage with stem lesions	Percentage with tuber infection	Marketable tubers			
							Yield per acre in bushels	Increase over diseased untreated check		
								Bushels	Percentage	Odds[2]
Clean, Dipdust 1–20 dip	W	98.8	86	6	6	28	374.2 ± 7.1	74.2 ± 8.8	24.7 ± 2.9	M[3]: 1
Clean, Dipdust 1–20 dip	C	98.1	88	40	14	42	369.0 ± 6.6	69.0 ± 8.4	23.0 ± 2.8	M: 1
Clean, corrosive sublimate 1–1000 1½ hr.	W	98.1	87	8	2	27	368.2 ± 7.0	68.2 ± 8.7	22.7 ± 2.9	M: 1
Clean, Semesan Bel 1–20 dip	C	92.2	89	34	37	39	366.2 ± 6.6	66.2 ± 8.4	22.1 ± 2.9	M: 1
76 Bel 1–40 dip	W	98.9	81	8	4	25	362.2 ± 5.4	62.2 ± 7.6	20.8 ± 2.5	M: 1
Clean, check, not treated		99.2	83	10	14	35	361.8 ± 5.2	61.8 ± 7.4	20.6 ± 2.5	M: 1
Formaldehyde 1–240 2 hr.	W	99.6	84	14	14	38	359.0 ± 7.0	59.0 ± 8.7	19.7 ± 2.9	434782: 1
Corrosive sublimate 1–1000 1½ hr.	W	96.5	80	2	10	27	358.6 ± 5.7	58.6 ± 7.7	19.5 ± 2.6	M: 1
Clean, Semesan Bel 1–20 dip	W	100.0	88	4	30	29	358.2 ± 7.2	58.2 ± 8.9	19.4 ± 3.0	M: 1
Dipdust 1–20 dip	C	98.1	81	16	38	70	351.0 ± 5.5	51.0 ± 7.6	17.0 ± 2.5	434782: 1
77 Bel 1–20 dip	W	98.8	78	2	35	60	347.0 ± 5.3	47.0 ± 7.5	15.7 ± 2.5	19230: 1
Dipdust 1–20 dip	W	99.2	76	14	45	67	340.3 ± 5.4	40.3 ± 7.5	13.4 ± 2.5	1350: 1
Bayer Special 190 1–20 dip	W	98.8	73	12	37	64	339.9 ± 4.2	39.9 ± 6.7	13.3 ± 2.2	19230: 1
76 Bel 1–40 dip	C	98.8	72	2	8	30	335.1 ± 7.7	35.1 ± 9.3	11.7 ± 3.1	95: 1
Bayer Special 181 1–40 dip	W	98.1	69	4	26	68	329.5 ± 7.0	29.5 ± 8.7	9.8 ± 2.9	45: 1
Semesan Bel 1–20 dip	C	95.0	74	60	33	61	325.9 ± 5.7	25.9 ± 7.7	8.6 ± 2.6	32: 1
Formaldehyde 1–120 52° C. 3 min.	W	98.5	60	4	16	36	317.9 ± 6.8	17.9 ± 8.6	6.0 ± 3.0	4.6: 1
Semesan Bel 1–20 dip	W	99.6	63	14	56	88	317.5 ± 5.1	17.5 ± 7.3	5.8 ± 2.4	8.5: 1
Diseased, water, dip	W	95.6	65	48	62	66	310.3 ± 3.3	10.3 ± 6.1	3.4 ± 2.0	3: 1
Diseased, check, not treated		96.5	57	38	79	83	300.0 ± 5.2			

[1] W = whole; C = cut.
[2] Significance is shown by odds of over 22 to 1.
[3] M = a million or more.

Seed-piece decay. Seed-piece decay ranging from 2 to 60 per cent appeared in the different lots. The lots treated with 76 and 77 Bel dip and with corrosive sublimate each developed only 2 per cent of decay, while the nontreated diseased and water-dipped diseased controls showed 38 and 48 per cent of decay, respectively. Cut seed treated with Improved Semesan Bel developed 60 per cent decay, suggesting injury as well as ineffective control of organisms responsible for decay. The highest percentage of seed-piece decay appeared on treated cut seed and on the diseased controls, indicating possible treatment injury as well as injury from organisms harbored in the soil or on the tuber.

Stem lesions. Stem infection ranged from 4 to 56 per cent for the treatments, while the highest infection, 79 per cent, appeared on the diseased control. The best control was obtained with 76 Bel which apparently was slightly more effective than corrosive sublimate. The total injury from lesions was most severe in the lots showing the highest percentage of infected stems. As in previous seasons, stem infection was closely correlated with infection of seed pieces, although the relatively high stem infection in the clean controls indicates either some undetected seed-piece infection or soil infestation.

Each of 12 treated lots developed 1 per cent or less of black-leg hills, while the remaining six treated lots showed from 1 to 3 per cent black leg, the highest percentage appearing in the lot treated with hot formaldehyde. No black leg occurred in the diseased control. Although present, black leg was not abundant enough to lead to definite conclusions.

Tuber infection. The diseased lots showing the lowest tuber infection, 25 and 27 per cent, were treated with 76 Bel and corrosive sublimate, respectively. The highest percentage, 88, of infected tubers appeared in the lot treated with Improved Semesan Bel, which was the only one showing more infection than the diseased control. The relatively high percentage of infected tubers in the clean treated and clean control lots indicates some infection from the seed pieces or from the soil. Some correlation appears between stem lesions and tuber infection.

Yield. Significant increases in yield were obtained from all the treatments, with the exception of Improved Semesan Bel used on whole tubers, which yielded only slightly more than the diseased control. The highest increases in yield in the diseased lots were obtained with 76 Bel, cold formaldehyde, and corrosive sublimate. Hot formaldehyde gave fair control of Rhizoctonia infection but increased the yield only 6 per cent. This is barely significant. Possibly this treatment was injurious to the seed pieces and thus inhibited the yield as much as did injury from Rhizoctonia. Clean seed treated with Dipdust, corrosive sublimate, and Semesan Bel dip produced slightly higher yields than the highest yielding, treated, dis-

eased lots and the clean control. The lowest yielding lots were also associated with the lowest relative vigor.

As in previous seasons, the greatest increases in yield were not invariably obtained from the lots showing low infection with Rhizoctonia, a fact which suggests that possible treatment injury may reduce yield more than does Rhizoctonia infection.

DISCUSSION AND CONCLUSIONS

Recent results from investigations outside of Maine.—The writers have made a survey of results reported from recent seed-potato-treatment experiments, especially with reference to the effects upon Rhizoctonia and yield. In view of the nature of the problem and of the results, it seems desirable to present here only the conclusions derived from this survey.

The results of the various recent investigations on potato treatment for Rhizoctonia show that corrosive sublimate is one of the most effective treatments for controlling this disease. However, in some cases corrosive sublimate has not been significantly more effective in killing Rhizoctonia sclerotia than formaldehyde or certain of the organic-mercury products.

The recent evidence on yield obtained with seed-potato treatments by investigators in different localities indicates that significant increases in yield resulted in some localities, while slight to significant decreases in yield appeared in the same season in other localities. Moreover, in the same locality increases in yield resulted in one season, while in another decreases in yield appeared in connection with the same treatment. Comparative seed-potato treatments with corrosive sublimate, hot formaldehyde, and organic-mercury fungicides conducted in the principal potato-producing States indicated that certain organic-mercury preparations gave more uniform results on yield than either corrosive sublimate or hot formaldehyde.

The evidence that certain treatments giving the best control of Rhizoctonia failed sometimes to increase the yield indicates that injurious effects on the tubers from treating may inhibit yield more than Rhizoctonia.

This brief summary from a review of literature shows that the results obtained elsewhere lead to the same general conclusions as may now be drawn from the results in Maine.

Results in Maine. Treating Rhizoctonia-infected potatoes with corrosive sublimate, formaldehyde, and certain organic-mercury fungicides effectively inhibited Rhizoctonia. However, the percentage of badly infected tuber progeny from the nontreated diseased controls frequently was so low that the appearance of the entire lot was not greatly affected. Under such conditions, it is apparent that the advisability of treating seed potatoes could be questioned unless significant increases in yield could be shown. From the results on control and yield obtained in 1925 one might

have doubted the advisability of seed-potato treatment, particularly of tubers relatively free from Rhizoctonia sclerotia.

However, the results obtained from the experiments in 1926 to 1928 show that under other conditions certain treatments effected significant increases in yield and, with a few exceptions, increased the percentage of clean tubers.

Nontreated clean tubers, which did not manifest Rhizoctonia sclerotia, frequently produced cleaner tuber progeny and greater increases in yield than treated diseased tubers, which were uniformly covered with sclerotia. These results indicate that seed-piece-infection was correlated with infection of progeny and with yield. A similar correlation appeared between Rhizoctonia infection of seed pieces and of stems in the absence of soil infestation or in soils apparently slightly infested with Rhizoctonia.

The evidence that certain treatments inhibited seed-piece decay, which apparently affected the yield in 1927, emphasizes the importance of observations on the condition of the seed piece in the soil and on the vigor of the plants. It is probable that Rhizoctonia at times may have been wrongly regarded as the primary cause of reduced yields, unless this disease should be related in some manner to conditions favoring seed-piece decay. It is interesting to note that in 1927 all the seed pieces in the nontreated Rhizoctonia control manifested different stages of decay involving in severe cases the entire seed piece when the plants were about 6 inches above ground, while no decay occurred in several of the treated lots. The greater number of treatments showing significant increase in yield in 1927 than during the two previous seasons apparently indicates that seed-piece decay may have been one of the contributing factors.

Results on control of Rhizoctonia and on yield obtained from 1925 to 1928 with some organic mercuries and with corrosive sublimate and formaldehyde are represented in figure 1. The corrosive sublimate and formaldehyde treatments were applied to whole tubers. In 1925 and 1928 the organic-mercury treatments represented in figure 1 were applied to whole tubers, while, in 1926 and 1927, these treatments were used on cut tubers. Since the differences between treated cut and treated whole tubers were not invariably significant regarding yield or control, these factors do not materially affect the comparison of the results. Certain modifications of the organic-mercury preparations made by the manufacturers during the four seasons prevent adequate season-to-season comparisons of these results. However, the effectiveness of these products can be compared with that of corrosive sublimate and formaldehyde during the same season.

Although our experience with seed-potato treatments conducted on the same farm during four seasons shows that Rhizoctonia can be inhibited and that the yields can be maintained as well as increased, the results ob-

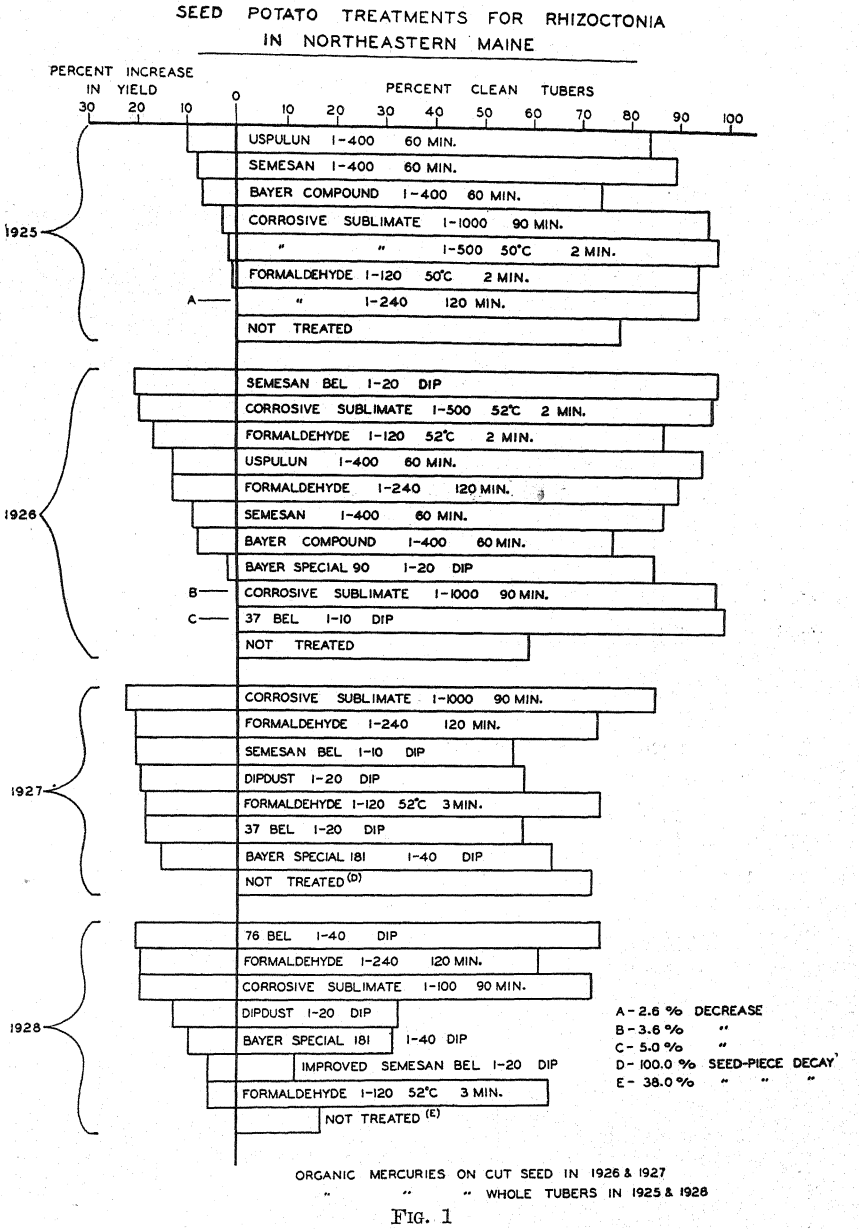


FIG. 1

tained here must not be regarded as criteria of what may occur under different conditions. Treating small lots of potatoes, storing them in crates favorable to drying after treating, and planting the seed pieces by hand

may be conducive to more nearly optimum conditions than prevail in general practice, especially where large lots of potatoes are treated. Furthermore, variations in variety, dormancy of tubers, nature and extent of diseases, moisture, temperature, and soil may greatly influence the results. Moreover, the evidence that, under certain conditions, treating sprouted tubers, delayed drying of tubers after treating, and exceeding the optimum concentration and time of treatment, potatoes may be injured by treating emphasizes the importance of treating seed potatoes under as nearly optimum conditions as possible. Additional investigations covering various factors, which may influence the results of treating potatoes, may contribute to improved methods of conducting seed-potato treatments.

Inconsistent results on yield emphasize the importance of additional investigations relating to various factors which are involved in seed-potato treatments. It will suffice to mention improvement in treating methods and in conditions of storing and handling treated potatoes, stage of dormancy and maturity of tubers, variations in susceptibility of different varieties to treatment injury, modifications of disinfecting products making them less injurious to the tubers but maintaining their toxic effects on the pathogenic organisms, the nature and extent of the diseases to be controlled, and the effect of soil, temperature, and moisture on host and pathogene.

SUMMARY

1. In the seed-potato treatments conducted from 1925 to 1928 on Aroostook Farm, Maine, consideration was given to replication of plots, seed-piece decay, stand (percentage of plants emerging), vigor, stem lesions, sclerotial infection of tubers by Rhizoctonia, and yield. The treated lots were of the Irish Cobbler variety, were mostly of one commercial stock, and were planted in Caribou loam.

2. Seed treatment with most of the materials reduced seed-piece decay, improved the stand, increased the vigor, inhibited the formation of stem lesions and of tuber-borne sclerotia by Rhizoctonia, and increased the yield. However, the same treatment has varied in its effects from one season to another, giving sometimes significant and sometimes nonsignificant differences or even undesirable results such as seed-piece injury or yield reduction.

3. Corrosive sublimate was one of the best treatments for controlling Rhizoctonia and in two seasons effected the highest increase in yield. However, the increased yields obtained with corrosive sublimate were not always significantly greater than those obtained with formaldehyde and with certain organic-mercury compounds. Hot corrosive sublimate gave as effective control of Rhizoctonia as cold corrosive sublimate. Hot formalde-

TABLE 5.—*Arrangement of seed-potato treatment plots in 1927*

J																												
		15	13	17	11	19	9	20	7	18	5	16	3	14	1	12	2	10	4	8	6				20	10	1	11
I		3	1	5	2	7	4	9	6	11	8	13	10	15	12	17	14	19	10	20	18				19	9	2	12
H		14	16	12	18	10	20	8	19	6	17	4	15	2	13	1	11	3	9	5	7				18	8	3	13
G		9	7	11	5	13	3	15	1	17	2	19	4	20	6	18	8	16	10	14	12				17	7	4	14
F		16	18	14	20	12	19	10	17	8	15	6	13	4	11	2	9	1	7	3	5				16	6	5	15
E		2	4	1	6	3	8	5	10	7	12	9	14	11	16	13	18	15	20	17	19				15	5	6	16
D		13	11	15	9	17	7	19	5	20	3	18	1	16	2	14	4	12	6	10	8				14	4	7	17
C		10	12	8	14	6	16	4	18	2	20	1	19	3	17	5	15	7	13	9	11				13	3	8	18
B		17	15	19	13	20	11	18	9	16	7	14	5	12	3	10	1	8	2	6	4				12	2	9	19
A[1]		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20				11	1	10	20
	Buffer row																					Buffer row			K	K	L	L

[1] A to L represent replications of which A to J were used for yields and K and L for observations on stem lesions and seed-piece decay.

The numbers represent the different treatments.

hyde controlled Rhizoctonia as well as cold formaldehyde, but was less effective than corrosive sublimate.

4. Among the organic-mercury fungicides 76 Bel dip, used only in 1928, was as effective for Rhizoctonia control and for increase in yield as corrosive sublimate. Although Dipdust (1–20) used in 1927 and 1928 gave consistent increases in yield, it did not inhibit Rhizoctonia as well as corrosive sublimate and formaldehyde. Semesan Bel (1–10) used in 1926 and 1927 gave about the same results as Dipdust (1–20). Improved Semesan Bel (1–20) used in 1928 apparently was less effective than Semesan Bel (1–10).

5. Seed treatment was most effective on seed tubers infected with Rhizoctonia. Beneficial results were also secured in some cases by seed treatment of apparently disease-free tubers.

6. Seed-piece decay was inhibited as well by treating whole tubers as by treating cut seed. Seed-piece decay sometimes reduced the stand, vigor, amount of Rhizoctonia infection of stems and tubers, and yield.

7. Tuber infection by Rhizoctonia sclerotia was not necessarily abundant enough to detract from the appearance of the crop even when grown from nontreated infected seed.

8. As indicated above, the yield was influenced by various conditions of the test. It was increased most by Rhizoctonia control in 1928; but, in 1927, was increased most by control of seed-piece decay.

9. Recent tests conducted outside of Maine and those reported here, agree in respect to the variable effect of seasonal or climatic factors, and that the same treatment may vary in results with the locality and type of soil.

10. The development of disinfecting products simple in application, noninjurious to the seed potatoes, and effective in disease control appears essential for insuring the widest application of seed-potato treatments. Noninjurious instantaneous dip treatments perfected so that they will control Rhizoctonia and similar tuber-borne maladies more effectively than the present products seem to offer possible prospects for improving seed-potato treatment.

TWO PHYSIOLOGIC FORMS OF *USTILAGO STRIAEFORMIS* (WESTD.) NIESSL

W. H. DAVIS

Ustilago striaeformis is a smut organism which commonly parasitizes many of our common grasses. Mycologists and pathologists have based the classification of this organism on the following morphological characters which appear to be fairly constant for the smut collected from many hosts: Echinulate chlamydospores (spores); similarity in spore measurements; germination of the chlamydospores as in the genus Ustilago; and similarity of the symptoms which are indicated by long, black stripes containing chlamydospores principally located in the leaves and stems of the hosts.

Granting that there is but one morphological species parasitizing the different hosts, the physiology of the fungus or the problem of physiologic forms (physiologic races or physiologic species) has remained unsolved and this investigation was undertaken partially to solve the problem through an attempt to answer the following questions:

1. Are there physiologic forms of *U. striaeformis*?
2. Specifically, will germinated chlamydospores removed from timothy *Phleum pratense* L., infect seedlings of redtop, *Agrostis palustris* Huds.?
3. Will germinated chlamydospores removed from redtop infect timothy seedlings?
4. Will reciprocal inoculations show infections similar to inoculations within these two host species?
5. Are the morphological characters of the smut organism on different hosts similar?

COMMON NAME

Leaf smut of timothy is the common name by which this disease has generally been known on each of the different grass hosts in America. This common name was first employed by Trelease (7) in 1882. Pammel (6), in 1901, reported it as *timothy smut* but Rostrup, in Denmark, (Lind, p. 267) spoke of it as grass smut (*"Grassernes Stinkbrand"*). This smut has been reported on 23 different genera and 40 species of grasses, other than timothy, from Europe, America, and Australia. Furthermore, the writer (1) has shown that the organism is not wholly confined to leaves but is systemic, as hyphae and chlamydospores have been located in rhizomes, culms, leaves, and floral parts of grasses. Thus the usage of leaf smut and its association with timothy alone are misleading. So, to conform with the facts, the common name *leaf smut of timothy* should be discontinued and *striped smut of grasses* be substituted.

HISTORICAL

Osner (5) was the first to report results of inoculations with chlamydospores of *Ustilago striaeformis* but he did not report reciprocal and multiple inoculations, the results of which are necessary to determine physiologic-form specialization. Furthermore, infection studies could not have been properly conducted since the proper conditions for germination of the chlamydospores, the locations of the infection area (seedling infection), and the life history of the organism were then unknown.

Lind (3) stated: "It has not yet been proved whether all the said forms are really to be joined into one species, still such morphological differences as might condition a different species do not occur."

Liro (4) has presented a review of the classification of this organism and has shown the need for an investigation of physiologic-form specialization. A translation follows: "It is difficult to recognize the various species of this fungus and one cannot help but wonder whether the mycology of *Ustilago striaeformis* has definitely given so much deception that it will take considerable labor and pains before we can satisfactorily learn to recognize the sub-species of this group-species. But first of all, the biology of the species, concerning which very little is known in literature, must be thoroughly investigated."

FIELD OBSERVATIONS ON THE HOSTS

During the past eight growing seasons, 1920–1927, inclusive, the writer made field observations and collections in Wisconsin, Iowa, Illinois, New York, Pennsylvania, Maine, New Hampshire, Vermont, Massachusetts, and Connecticut. These observations were made specifically to determine the existing host range and to collect spore materials for experimentation. They may be partly summarized as follows:

1. In the various meadows, pastures, and lawns, timothy and redtop plants, together with those of other grasses, had been growing in contact with one another for several years. The species of plants smutted may be grouped:
   - A. Timothy infected with smut, but all the redtop and other grasses smut-free.
   - B. Redtop infected, but the timothy and other grasses smut-free.
   - C. Smutted timothy and redtop in the same field with other species of healthy grasses.
   - D. Timothy and redtop smut-free, but other species of grasses smutted.
   - E. No smutted grass to be found in the plat.
2. In general, only one grass host was severely parasitized at a given station or plat. If other smutted grasses were present, the plants were generally scattered and in the minority. From these generalizations,

it seemed that there were some evidences to show the presence of physiologic forms in the field.

MATERIALS AND METHODS

The timothy and redtop seeds, from which the seedlings were incubated for inoculation, were collected from the following sources:

1. A seed firm at Boston; 2. White mountain timothy from New Hampshire; 3. An Iowa grower of timothy seed; 4. Collections from fields in Wisconsin, Massachusetts, and Connecticut.

The redtop was *Agrostis palustris* Huds. (*A. alba* L.). Other varieties of this species were not employed in the seed collections.

All seeds had been stored for one or more years before they were germinated for experimental purposes. It has been shown (1) that, under optimum conditions, the chlamydospores of *U. striaeformis* seldom, if ever, remain alive more than one year. Thus, it seemed unnecessary to sterilize the seeds, as the seedlings were considered free from infection by any chlamydospores which might have remained attached to them. However, checks were prepared by transplanting uninoculated seedlings incubated from each of the seed collections.

The spore materials were collected from twelve different stations in four different States, but, as shown in table 1, most of these stations were located in Massachusetts. This range was considered sufficiently wide in scope to include any physiologic forms of the fungus that might parasitize either host. Furthermore, spore materials were collected in 1922 and 1924 from grass plants previously removed from fields in Wisconsin and transplanted in the pathological garden at the Massachusetts Agricultural College. When collected, most of the spore materials were within living, green leaves and stems of the hosts. When each sample of spore material was removed from the host, it was placed in a paper packet which was folded and properly labeled. Special care was taken to prevent the spores in one packet from being transferred to another. The collections were made separately and the packets not allowed to come in contact with each other. Spore materials were removed also from artificially inoculated plants in the greenhouse by employing inocula removed from the same host species. These are indicated in table 1 by the numbers 4, 6, 8, and 11.

Most of the spore materials were transferred from the packets to damp filter paper within covered jars and stored at room temperature, 20° C. The jars consisted of Petri dishes and glass-stopper bottles of various sizes. The filter paper in these bottles was kept moist by occasionally adding a small amount of cool, boiled tap water. Likewise, fifty of these spore materials were prepared, but inocula were used from only 12, as indicated in table 1. The others were too contaminated or the spores did not germinate

TABLE 1.—*A table showing the localities, dates, and hosts from which Ustilago striaeformis was collected for inoculum. Also, the dates and percentages of spores germinated in these collections*

Inoculum number	Host	Collection		Germination		Remarks
		Locality	Date	Date	Per cent	
1	Timothy	Windsor, Conn.	7– 6–24	2– 3–25	50	Dead infected leaves and stems stored
2	Timothy	Amherst, Mass.	5–26–26	2– 7–27	88	Infected young plants
3	Timothy	Madison, Wis.	5– 4–25	2–26–26	90	Smutted plants transplanted from Wisconsin
4	Timothy	Madison	5–28–26	10–30–26	90	From plants artificially inoculated in the greenhouse
5	Timothy	Windsor	6– 6–25	2–26–26	85	Collected from two stations
6	Timothy	Amherst	6–25–26	10–30–26	50	From inoculated plants
7	Timothy	Amherst	5–28–26	10–30–26	75	
8	Timothy	Amherst	5– 8–26	10–30–26	99	From artificially inoculated timothy plants
9	Timothy	Ithaca, N. Y.	8–20–26	10–30–26	50	Old infected leaves dried 30 days before storage
10	Redtop	Windsor	7– 6–24	2– 3–25	80	Spore materials stored on the surface of soil
11	Redtop	Amherst	6–23–26	11–20–26	99	Artificially inoculated redtop
12	Redtop	Amherst	6–30–26	2– 3–27	99	Collected from the field
13	Redtop	Madison	6–31–24	1–13–25	80	Smutted plants transplanted from Wisconsin
14	Redtop	Amherst	10–10–24	6– 1–25	85	Infected seedlings stored

sufficiently for investigational purposes. It would seem that the geographic distribution of these 12 spore materials was sufficient to include any widely distributed physiologic form of this smut. The dates on which the spore germination tests were made, together with the percentage that germinated, are also shown in table 1. The methods employed in the inoculations were those which have already been described by the writer (2). Both germinated and ungerminated chlamydospores were placed on the coleoptiles of seedlings submerged in water. Furthermore, seedlings grown from hull and hulled seeds were employed, but the final results sought were the same, regardless of the hulling. However, a higher percentage of infected plants was obtained by removing the hulls in one inoculation (Table 2, A). Seedlings of different ages and lengths of coleoptile were inoculated. The age of the seedlings was considered to be the number of days between the date on which the seeds were set to germinate in distilled water and the date their seedlings were inoculated. This period, together with the operation of other factors such as heat, light, and viability of the seeds, determined the length of the coleoptile. Seedlings with coleoptiles longer than 4 mm. were not inoculated.

Seedlings were never planted twice in the same greenhouse soil for fear that some of the ungerminated chlamydospores falling from seedlings of a previous planting might infect subsequent ones.

The water-holding capacity of the soil could not be satisfactorily controlled, but an effort was made to retain it at 30 per cent of its holding capacity as figured on a dry basis.

By observing the above precautions, the conditions for infection, together with the purity of viability of the inoculum and seed, seemed sufficient for experimental purposes.

INOCULATIONS

Several preliminary tests were made in which inoculated timothy and redtop seedlings were transplanted in plats located in the pathological garden (table 2, series A, I, J, K). As the results of these inoculations in the open were afterwards verified by others in greenhouse beds, the remainder of the work was performed in the greenhouse, where some of the environmental conditions could be controlled and the plants were little influenced by the change of seasons.

The first observation of the inoculated seedlings was generally recorded about six weeks after they had been transplanted. Thereafter, observations and records were made fortnightly. In some series, the infected plants were removed as soon as they were observed; in others, they were marked by placing wooden labels in the soil at their sides. However, only one case was recorded in which infection was observed before five weeks

had elapsed after the inoculations. It seemed necessary, though, to continue the observations until most of the inoculated plants had developed stools and floral parts, for it was often observed that only stools and floral parts showed symptoms of the disease.

A symptom previously unknown to the writer developed in several inoculated timothy plants. The plants were stunted and the stems twisted similar to those of wheat when infected by the wheat nematode, *Thylenchus tritici* (Steinbuch) Bastian. In one case, mycelium of the smut was located in these plants and chlamydospores had formed, but no nematodes could be found.

CHLAMYDOSPORE MEASUREMENTS

Davis (1) reported that the chlamydospores from timothy and redtop were about the same length, but those from redtop were slightly wider (timothy, 8.4 x 11 microns; redtop, 9.3 x 11 microns). Fresh chlamydospores were collected in Wisconsin, Connecticut, Massachusetts, New Hampshire, and New York and measured during the spring, summer, and fall for two years. Also, chlamydospores from each inoculum indicated in table 1 were measured. No method was found whereby the chlamydospores of either host could be differentiated by relying on morphologic characters alone. More often, the chlamydospores of redtop were the broader and of a deeper or intenser color. Sometimes, smutted redtop collections could be differentiated from those of timothy by the deep chocolate color of the chlamydospores. The sizes of chlamydospores varied with the seasons, as those from both hosts were somewhat larger in the early spring and late fall than in midsummer.

In each series, the number of seedlings inoculated and the percentage of infected plants are recorded in table 2. In series A, 220 timothy plants and 204 redtop seedlings, or a total of 424 seedlings, survived after being inoculated and transplanted. In series E, each of the five lots was inoculated with chlamydospores from a different source and 1508 seedlings survived. In B and C, timothy inoculum number 5 from Connecticut infected timothy seedlings incubated from seeds collected in Wisconsin and Connecticut; timothy inoculum 7 from Amherst infected seedlings incubated from seeds collected in Massachusetts and New Hampshire; inocula 11 and 12 from redtop collected at Amherst infected seedlings incubated from redtop seeds collected in Massachusetts, New Hampshire, and Connecticut.

In series W, it is to be noted that timothy inocula collected at Madison, Amherst, and Ithaca infected timothy seedlings from seeds collected in Massachusetts; also, that inoculum from redtop collected at Amherst infected seedlings from redtop seeds collected in Iowa. Likewise, various other combinations in the inoculations are shown in table 2.

TABLE 2.—*Table showing the sources of inocula and seed; dates of inoculation and planting, together with percentages of infection in 16 series of inoculated timothy and redtop seedlings*

Series	Source of inoculum	Source of seed	Date inoculated	Date planted	Seedlings inoculated		Percentage infected		Remarks
	(Timothy)				Timothy	Redtop	Timothy	Redtop	
A	Ser. 1, Conn.[1]	Iowa	6– 8–25	6–22–25	220	204	18	0	Preliminary test
B	S. 5, Conn.	Wis.	2–24–26	3– 2–26	48	60	12	0	Duplicated the following week
C	S. 5, Conn.	Conn.	3–18–26	3–26–26	161	119	10	0	
D	S. 3, Wis.	Iowa	3–28–26	4– 3–26	301	219	10	0	Duplicated with T.5 20 per cent infection
E	S. 4, 6, 7, 8, 9, Wis., N. Y., Mass.	Mass.	11– 3–26	11–10–26	806	702	9	0	Each showed infections
F	S. 9, N. Y.	N. Y.	2– 7–27	2–14–27	1437	219	20	0	
G	S. 4, 7, 2, Wis., Mass.	N. H.	2– 7–27	2–14–27	1337	711	8	0	Each showed infections
H	None, Checks	Each of the above	Same as Ser. A to G	Same as above	About 100 each	About 100 each	0	0	Checks all free from smut
	(Redtop)								
I	S. 10, Conn.	Wis.	6– 8–25	6–22–25	62	46	0	9	Preliminary test
J	S. 14, Mass.	Iowa	6– 8–25	6–22–25	24	84	0	37	Preliminary test
K	S. 13, Wis.	Iowa	2–13–25	2–21–25	44	60	0	12	Preliminary test
L	S. 11, Mass.	Conn.	11– 3–26	11–10–26	163	139	0	10	
M	S. 12, Mass.	Mass.	2– 6–27	2–14–27	137	433	0	9	
N	S. 12, Mass.	N. Y.	2– 7–27	2–14–27	110	99	0	11	
O	S. 11, Mass.	N. H.	2–13–27	2–20–27	92	166	0	18	
P	S. 12, Wis.	N. Y.	2–13–27	2–20–27	73	161	0	33	

[1] S. 1 refers to series 1 as shown in table 1, in which the chlamydospores were collected in Connecticut.

The percentage of infected seedlings was computed from the number of seedlings of the susceptible host inoculated and not from all seedlings inoculated. As in series a, table 2, 424 seedlings were inoculated of which 220 were timothy and, of these 220, 36 or about 16 per cent were infected, but redtop showed no infection. Where several inoculations were made in one series (Table 2, series E), the percentage of infection expresses that part of all the inoculated plants of one host which were infected. From table 2 it is to be noted that the percentages of infection in the various inoculations varied from 8 to 37. This was probably due to a variation of uncontrollable factors, such as differences in the stages of the seedlings and viability of the chlamydospores which were unavoidably removed from different sori in the spore materials; variation of temperature, sunlight, moisture, soil reaction, and fertility. However, the percentages of infected plants were sufficient to show that when the timothy and redtop seedlings incubated from seed collected in Iowa, Wisconsin, Massachusetts, Connecticut, and New Hampshire were inoculated with viable chlamydospores collected from these two hosts in Wisconsin, Connecticut, New York, and Massachusetts chlamydospores from timothy infected only timothy and those from redtop infected only redtop.

REACTION IN AGRONOMIC STRAINS OF TIMOTHY

The foregoing experiments had shown physiologic forms in *U. striaeformis* on two hosts, but it would seem that further evidence were needed to answer the following questions: Are some agronomic strains of timothy susceptible to this smut fungus? Are they resistant? Are they immune? Are there physiological forms of the smut fungus parasitizing timothy?

The following collections of timothy seed were made, in addition to the numbers indicated in table 2: Wisconsin, 3; New York, 2; Massachusetts, 7. Also, C. H. Myers of Cornell University furnished seed of his agronomic strains: 4081, 4001, 4123, 4003, 4031, 4078, 4079, 4059, 1777, 1676.

The spore materials were those indicated in table 1 and others, making a total collection from stations in each State as follows: Massachusetts, 5; Connecticut, 2; New York, 2; Wisconsin, 2.

The methods of inoculation were those described in the foregoing pages of this paper. The agronomic strains were inoculated with but one sample of inoculum collected in each State. Infected plants were observed in each of the inoculations. The artificial infection varied from 70 to 1 per cent. The agronomic strains were arranged in the order of their resistance, beginning with the least resistant: 4079–R1; 1676–R2; 4123–R3; 4001–R4; 1775–R5; 4081–R6; 4003–R7; 4078–R8; 4031–R9; 4059–R10.

No agronomic strains of timothy were found immune from the spore forms of *U. striaeformis* employed and no physiologic strains of the fungus were detected parasitizing this host species.

DISCUSSION

It is possible that collections from stations of a wider geographic distribution, or in foreign lands, may reveal physiologic strains of *U. striaeformis* parasitizing timothy. One can conceive the insurmountable task before the investigator if all possibilities are removed. However, if such strains should be demonstrated in the future, the physiologic forms of this smut parasitizing timothy and redtop might better be known as *biotypes* and physiological races or forms applied to those forms of the smut fungus on a single host species.

SUMMARY

1. *Ustilago striaeformis* is a Latin binomial which has been assigned by mycologists and pathologists to a smut fungus parasitizing about 40 of our grasses. This classification was based on a similarity of the symptoms and chlamydospores as found on each of the hosts.

2. The common name of the disease, *leaf smut of timothy,* should be discontinued and *striped smut of grasses* substituted.

3. Lind and Liro stated that an investigation ought to be made to determine whether this smut was of many or of one physiologic form as well as morphologic species.

4. Field observations showed some evidences of physiologic specialization.

5. This investigation was concerned with two hosts, namely, timothy and redtop. After-ripened chlamydospores from each of these hosts were employed as inoculum.

6. Chlamydospores were collected from 7 stations in Massachusetts; 2 in Iowa; 3 in Wisconsin; 1 in New York; and 3 in Connecticut. These chlamydospores were after-ripened (incubated) from 2 to 9 months and tests indicated 50 to 99 per cent germination. Of the 50 spore collections thus incubated for experimental purposes, only 14 were usable when after-ripened. The geographic distribution of these 14 collections was considered wide enough to include any widely distributed physiologic form of the smut which might exist in our north-central and eastern agricultural districts.

7. The seedlings inoculated were incubated from seeds of timothy and redtop collected in Iowa, Wisconsin, New York, Massachusetts, Connecticut, and New Hampshire. The geographic distribution of the seeds was considered wide enough in scope to include any widely cultivated agronomic strain of timothy which might be immune from or decidedly resistant to this smut fungus.

8. Reciprocal inoculations and inoculations within these two host species were performed on seedlings.

9. From observations and data which were recorded in table 2, these statements seem warranted:

A. Chlamyspores removed from timothy seldom, if ever, infect redtop, but may infect timothy seedlings.
B. Chlamydospores removed from redtop seldom, if ever, infect timothy, but may infect redtop seedlings.
C. There are at least two physiologic forms of *U. striaeformis,* one parasitizing timothy and one parasitizing redtop.

10. No definite morphologic characters were found by which the chlamydospores of the timothy form could be differentiated from those of the redtop form. However, those from redtop may sometimes be distinguished by their deep chocolate color.

11. Agronomic strains of timothy were inoculated and none was found immune from the smut fungus, the inoculum of which was removed from timothy. No physiologic strains of the fungus were detected parasitizing timothy.

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STUDIES ON THE RELATION OF TEMPERATURE TO THE GROWTH, PARASITISM, THERMAL DEATH POINTS, AND CONTROL OF MYCOGONE PERNICIOSA[1]

EDMUND B. LAMBERT

INTRODUCTION

It has been known for more than a quarter of a century that the disease of cultivated mushrooms known in France as "la mole" and in the United States as "bubbles" is caused by *Mycogone perniciosa* Magnus. In congested centers of mushroom growing this disease has become a limiting factor and it is probably conservative to estimate the annual loss in Pennsylvania at 15 per cent of the potential crop, or about 1,500,000 pounds of mushrooms. Experiments and observations have been made to determine possible sources of inoculum and the efficacy of formaldehyde as a fumigant and a soil disinfectant (1, 2, 3, 4). But there have been no controlled experiments made to determine the effect of environmental factors on the development of the pathogene. Because of the importance of temperature in mushroom culture and the ease with which growers can control it in a modern mushroom house, the writer has made a series of experiments to determine the relation of temperature to the growth of Mycogone and its capacity for infection and also to determine its thermal death points when exposed for different lengths of time. It was found to be killed by prolonged exposure to moderately-high temperature. This suggested further experiments which were made to determine the feasibility of eradicating Mycogone from casing soil by means of the heat generated in mushroom houses during the final period of fermentation of the manure.

RATE OF GROWTH AND INFECTION

The temperature in mushroom houses during the period of sporophore formation is usually held between 10° and 20° C. (50° and 68° F.). However, during hot spells in late spring or early fall the grower is unable to control the temperature and it may remain as high as 21° to 24° C. (70° to 75° F.) for several days. Many growers have noticed that bubbles are most prevalent during or shortly after these hot spells and assert that they are never bothered with more than an occasional bubble in houses which have been kept below 13° C. (55° F.) after casing. To throw additional light on this question experiments were made under controlled conditions

[1] The writer wishes to express his appreciation to Dr. L. A. Hawkins, Mr. R. C. Wright, and Dr. J. I. Lauritzen for the use of constant-temperature rooms and incubators and to Mr. J. F. Brewer for the drawings and the photographs.

to determine the effect of temperature on the rate of growth of *M. perniciosa* in pure culture and on the infection of mushrooms growing at different constant temperatures in artificially-infested soil.

As in Smith's (3) cultures, the fungus grew most rapidly when subjected to temperatures between 20° C. and 28° C. (68° and 82° F.), which is above the normal range of temperatures for growing mushrooms. At 15° C. (59° F.) growth was very slow and at 10° C. (50° F.) there was only a trace of growth (Fig. 1). From this we may conclude that the

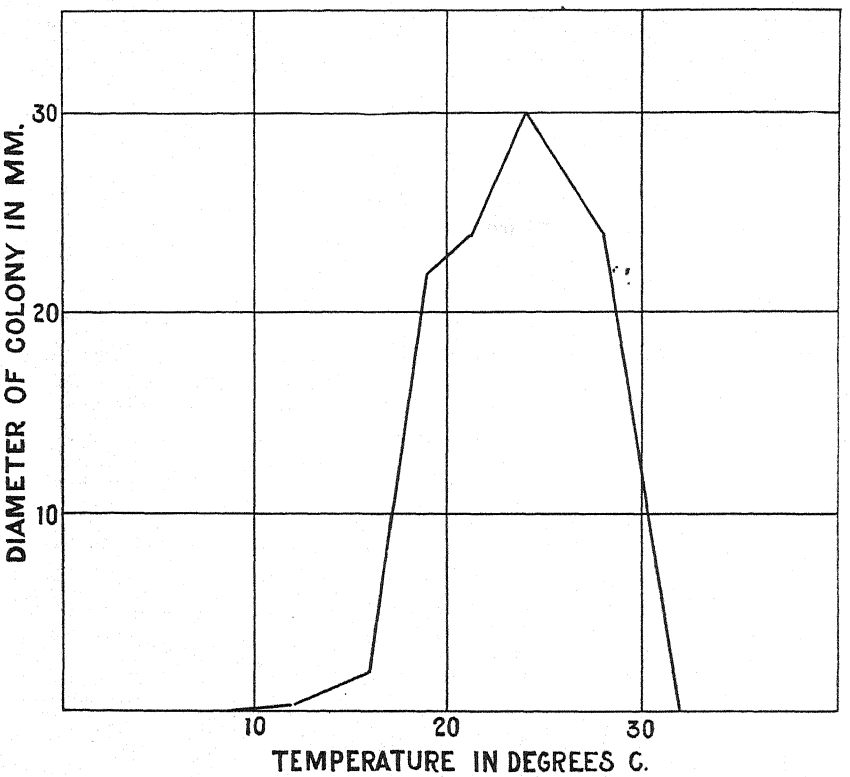


FIG. 1. The effect of temperature on the rate of growth of *Mycogone perniciosa* on Thaxter's agar; measurements were taken 7 days after inoculation.

organism spreads more slowly over the beds as the temperature approaches 10° C. (50° F.). But the formation of sporophores of *Agaricus campestris* also is markedly retarded as the temperature approaches 10° C., as shown in figure 2. Therefore, further experiments were made to determine if infection is diminished by low temperatures. In these experiments twelve mushroom cultures in aluminum cans were cased with artificially-infested moist soil and twelve were cased with Mycogone-free soil as checks. The

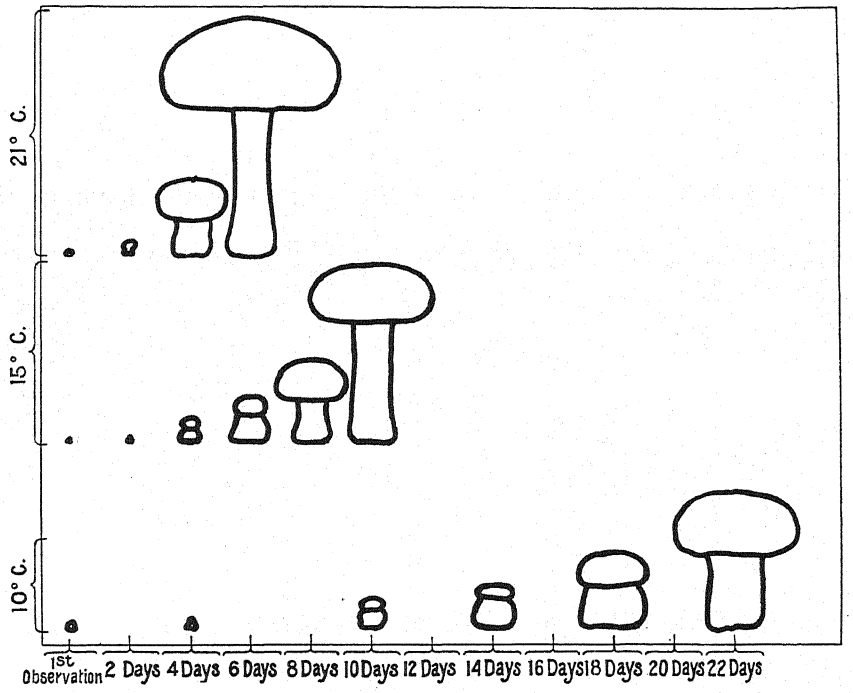


FIG. 2. The effect of temperature on the rate of growth of sporophores of *Agaricus campestris* var. "Snow White"; diagram based on the average of measurements taken from several specimens at each temperature.

cans were then placed in rooms in which constant temperatures were maintained at 21° C., 15° C., and 10° C. (70°, 60°, and 50° F.). There was 100 per cent infection in the eight cultures which were infested and held at 21° C. and at 15° C., while, at 10° C., there were more normal than infected mushrooms. There also was only a partial infection in cultures which had been 100 per cent infected at 21° C. and subsequently cleaned of bubbles and placed to grow again at 13° C. and 10° C. The cultures illustrated in figure 3 are typical of the results obtained. These experiments substantiate the observation of the growers that Mycogone is retarded to a greater extent than mushrooms by low temperatures and indicate that, even in the presence of an excess of inoculum, less bubbles may be expected at temperatures approaching 10° C. than at temperatures above 15° C.

THERMAL DEATH POINTS

According to the present practice, mushroom manure is fermented in such a way that all of the compost is subjected to comparatively high temperatures at one time or another. Outdoors, a large portion of the com-

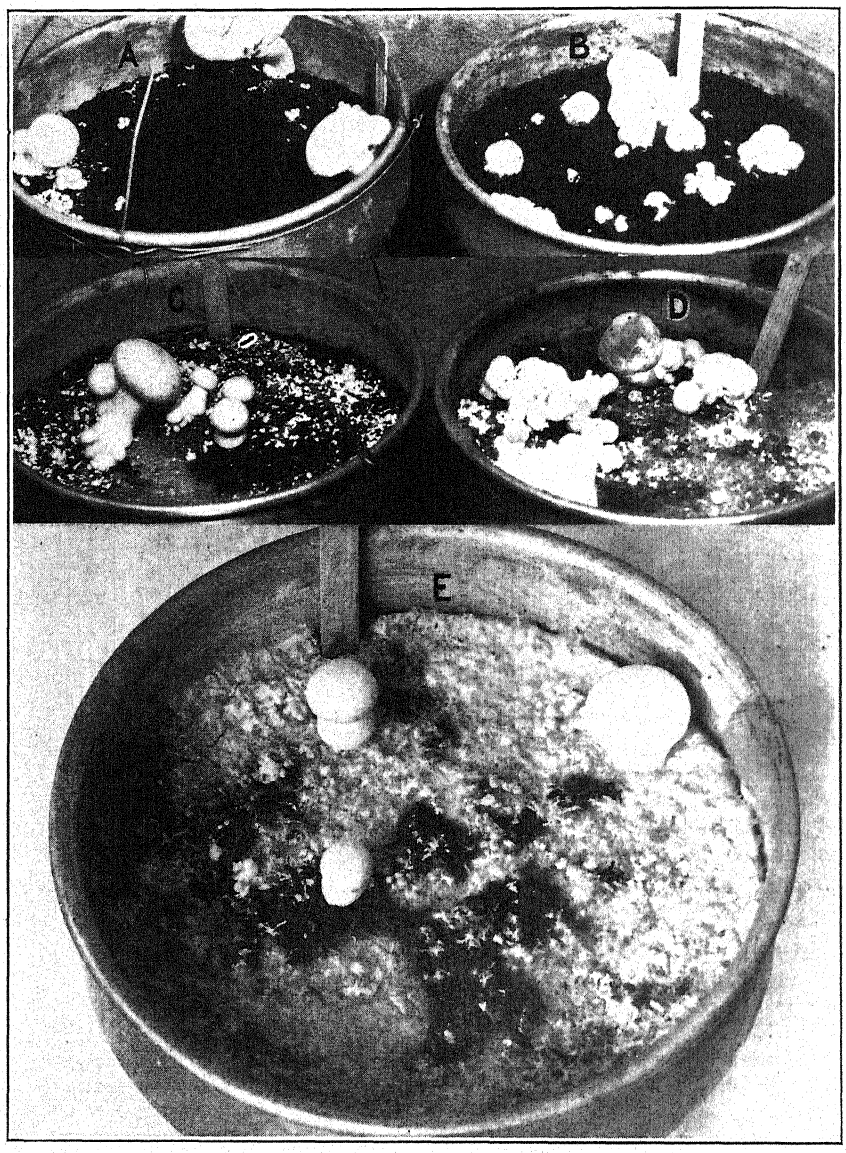


FIG. 3. The effect of temperature on the infection of *Agaricus campestris* growing in soil which had been generously infested with *Mycogone perniciosa*, strain 13A.—Culture A was cased with Mycogone-free soil and incubated at 70° F.; culture B was cased with infested soil and incubated at 70° F.; culture C was cased with Mycogone-free soil and incubated at 60° F.; culture D was cased with infested soil and incubated at 60° F.; culture E was cased with infested soil and incubated at 50° F.

post heap usually reaches temperatures of 60° to 75° C. (140° to 165° F.) for several hours at a time and inside of the house the beds are made with warm compost and the temperature continues to rise for a few days after they are made, often reaching 60° C. (140° F.). The air temperature inside the house, especially near the ceiling, may go as high as 50° C. (120° F.) for 24 to 48 hours. The question naturally arises, can *M. perniciosa* withstand these temperatures and persist in the manure? Several experiments were made to clear up this point. Preliminary tests indicated that pure cultures of Mycogone are more easily killed by high temperatures in sterile soil and sterile manure than in agar. Further experiments were therefore made to determine the temperatures at which cultures on agar in test tubes would be killed when exposed for different lengths of time. The transfers were made on Thaxter's agar from parent cultures about ten days old. As a precaution against drying out, large blocks of agar were transferred from the parent cultures and inverted on the fresh agar slants

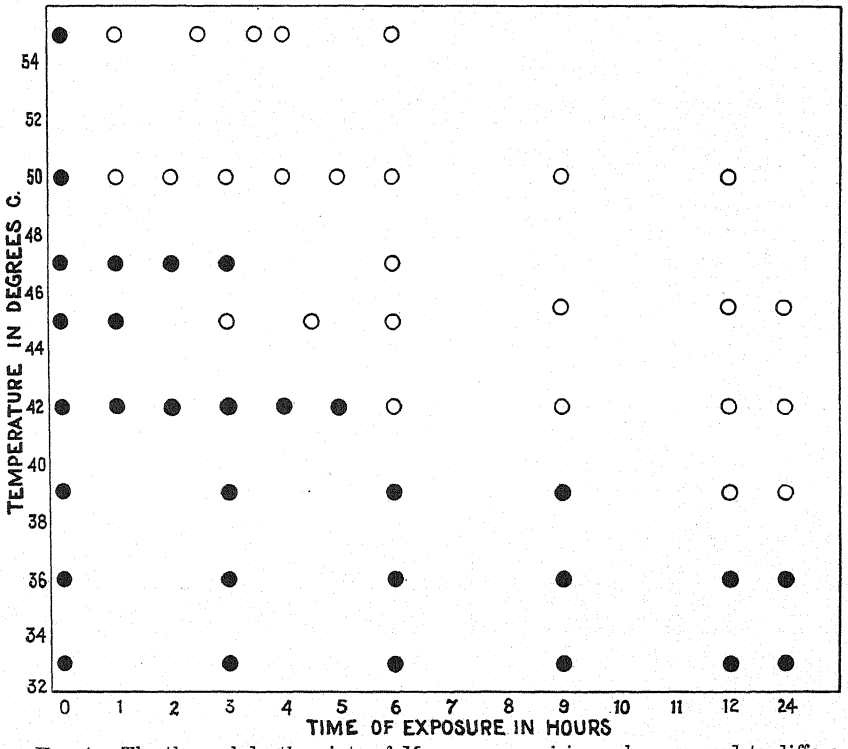


FIG. 4. The thermal death points of *Mycogone perniciosa* when exposed to different temperatures for different lengths of time; cultures were made on Thaxter's agar and the black dots indicate at least one live culture out of the four exposed, while the circles indicate all cultures dead.

so that an abundance of chlamydospores and Verticillium spores was buried in the agar. As an additional precaution beakers of water were placed in the incubator and at the higher temperatures the tubes were capped with tinfoil while they were heated. The results of this series of experiments are shown in figure 4. It is evident that Mycogone is quite sensitive to prolonged exposure to moderately-high temperatures. Under the conditions of these experiments vigorous cultures failed to survive exposure to temperatures no higher than 42° C. (108° F.) for periods of more than 6 hours. It therefore seems highly improbable that the pathogene can remain alive in actively fermenting manure.

EXPERIMENTS WITH HEATED SOIL

The results of the preceding experiments indicate that even the air temperature in the upper part of a well-managed mushroom house is high enough during the fermentation period to kill Mycogone spores. This suggested the possibility of utilizing this heat to eradicate soil infestation by placing the casing soil inside the mushroom house during the fermentation period. Several experiments were made to determine whether soil could be successfully treated in this way. In one series of tests, artificially-infested soil was subjected to temperatures of 52° C. and 45° C. (126° and 113° F.) for different lengths of time and subsequently used for casing mushroom cultures. The cultures were then placed under ideal conditions for the development of bubbles, the presence or absence of which was used as the criterion of presence or absence of the pathogene in the soil. The results of these tests, given in table 1, substantiate the conclusion drawn from data obtained with pure cultures on agar. Altogether they seemed to warrant making further tests in a commercial mushroom house.

TABLE 1.—*The effect of heating soil which has been artificially infested with Mycogone perniciosa (strains 48, 8, and 13A) on the amount of infection in cultures of Agaricus campestris, in which this soil was used for casing*

Temperature Centigrade	No. of hours treated	Number of mushrooms growing			
		Trial I		Trial II	
		Infected	Healthy	Infected	Healthy
52°	Check	6	0	4	0
	6 hours	2	29	0	10
	12 "	0	16	0	1
	24 "	0	10	0	6
45°	Check	33	0	4	0
	6 hours	1	11	1	4
	12 "	0	17	0	3
	24 "	0	1	0	22

In these experiments both naturally and artificially infested soils were subjected to the heat generated by fermenting manure.[2] The soil was placed in round metal pans 5 in. deep by 18 in. in diameter. These pans were set in a mushroom house over fermenting manure, with one edge touching the manure. The temperature of the soil rose to 131° F., about halfway between the air temperature (118° F.) and the manure temperature (140° F.). This temperature was maintained over night, about 14 hours. In the morning the house was opened to release the cyanide gas and the temperature immediately began to fall. By the next morning, when the soil was removed from the house, the temperature of the soil had dropped to 108° F. Four sections of bed were cased with this soil: Two with heated soil, naturally infested and artificially infested, and two with unheated soil, naturally- and artificially infested.

Six weeks later there was 100 per cent infection on the artificially infested check plot and on the naturally infested check plot there was about 80 per cent infection, while on both the plots cased with heated soil there was no trace of infection. The high percentage of infection in the check plot cased with naturally infested soil and the complete absence of infection in the corresponding heated plot were of particular interest since they offer at least one piece of evidence indicating that there are not likely to be more resistant spore forms in nature than there were in our artificially infested cultures.

DISCUSSIONS AND CONCLUSIONS

The results of the experiments presented in this paper tend to clear up several phases of the Mycogone problem: They offer a reasonable explanation for the severe outbreaks of bubbles which are so often associated with hot weather, they suggest a method of reducing the loss in infested houses, they indicate that manure probably is not a source of inoculum in mushroom houses in which there is an active fermentation during the final heat, and they suggest a simple and inexpensive method of eradicating *M. perniciosa* from infested-casing soil. The severe infection often appearing in houses which have been overheated during or following a hot spell seems to be due to the fact that the optimum temperature for the growth of *M. perniciosa* is higher than the normal range of temperatures for growing mushrooms, and the comparative scarcity of disease in cool houses seems to be correlated with the fact that under controlled conditions only sporadic infections develop at temperatures approaching 10° C. (50° F.), even in the presence of an abundance of inoculum in the soil. In

[2] The naturally infested soil was garden soil, heavily fertilized with spent mushroom manure for three years. The experiments were made in the houses of the Keystone Mushroom Company, Coatesville, Pennsylvania, and the facilities for making them were kindly placed at my disposal by Mr. L. F. Lambert and Mr. Charles H. G. Sweigart.

view of these facts it would seem advisable to keep the temperature below 13° C. (55° F.) in all houses known to be heavily infested.

Mycogone perniciosa has been shown to be quite sensitive to prolonged exposure to moderately high temperature. This indicates that it probably cannot withstand the temperatures developed in manure during active fermentation, especially during the final heat in the mushroom house. If the manure is left out of consideration, there is considerable circumstantial evidence which indicates that casing soil is the principal source of inoculum. Beach (1) came to this conclusion after three years' observation of conditions in Pennsylvania; and Smith (3), in England, and Beach have shown that formaldehyde can be used successfully to eradicate Mycogone from soil. In practice, however, this method has certain obvious disadvantages and it would seem to be simpler, safer, and less expensive for the average commercial grower to eradicate Mycogone from his casing soil by taking advantage of the low thermal death point of this fungus. The experiments outlined above indicate that usually high enough temperatures are generated in mushroom houses while the manure is going through its final heat. Of course, further work must be done before we can be sure that sufficient heat will be generated in all cases and that there are not heat-resistant strains of *M. perniciosa* in the soil. Perhaps it will be necessary to use artificial heat.

SUMMARY

1. Experiments were made to determine the effect of temperature on the growth of *Mycogone perniciosa* and on infection of mushrooms by this pathogene, the thermal death curve of the organism, and the possibility of controlling the bubbles disease by subjecting the casing soil to heat generated by fermenting manure.

2. The cardinal temperatures for the growth of the organism on Thaxter's agar are 8° C., 24° C., and 32° C. The most vigorous growth was made between 21° C. and 28° C., which is higher than the range of temperature in which mushrooms are usually grown under cultivation.

3. In experiments in which the relation of temperature to infection was tested there was 100 per cent infection in the cultures held at 21° C. and 15° C., but only an occasional diseased specimen appeared at 10° C.

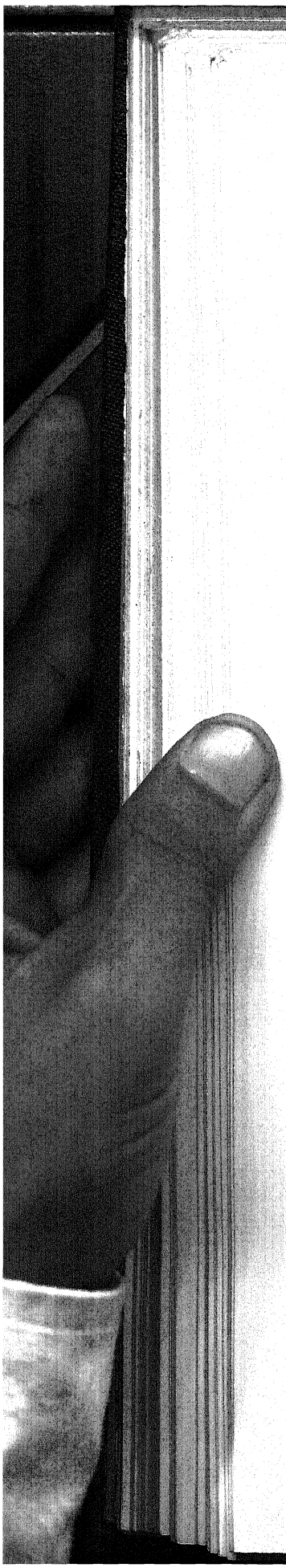
4. In agar cultures *M. perniciosa* was killed by exposure to temperatures of 42° C. (106° F.) or higher for 6 hours or more.

5. The bubbles disease did not develop in mushroom cultures cased with artificially-infested damp soil which had been subjected to temperatures of 45° C. and 52° C. for 12 hours and 24 hours; there were 100 per cent infection in the check cultures and an occasional infection in cultures exposed for only 6 hours.

6. The disease was controlled also by subjecting artificially infested and naturally infested casing soil to heat generated by the normal fermentation of manure in a commercial mushroom house.

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PATHOGENICITY OF *PESTALOTIA* SPP. ON RHODODENDRON[1]

RICHARD P. WHITE

Pestalotia macrotricha Kleb. and *Pestalotia rhododendri* Guba are common organisms on Rhododendron. They follow sun scald on leaves and young stems (Fig. 1, A. B1), where they cause dark brown, zonate spots, later becoming silvery and covered on the upper surface with acervuli. These symptoms also are found on leaves following injuries produced by the Rhododendron lace bug, *Stephanitis rhododendri* Horv., and other types of mechanical and insect injuries (Fig. 1, B2).

It has been shown by Guba (3) that these organisms have been erroneously referred to as *Pestalotia guepini* Desm. Van den Broek and Schenk (6, p. 327) picture this trouble on Rhododendron leaves and twigs and state that young plants may be killed to the ground by it. Clinton (1) also illustrates Pestalotia on Rhododendron leaves on plants imported from France and Holland, and Schmitz (4) discusses the disease as found on cultivated Rhododendron in this country. The photograph in the latter paper is not of such nature that the disease can be recognized. It is significant, however, in discussing the pathogenicity of the organism, that the author considers it secondary in nature to the attacks by aphids on young leaves early in the season.

Schmitz further states that "the usual methods of artificial inoculation were employed and positive evidence was obtained, showing that this species of Pestalozzia is parasitic in nature." His method of inoculation was to seal a glass ring on the upper surface of a leaf with paraffin and beeswax. Spore suspensions were then placed in the cell and a cover glass sealed over the top. This procedure is quite likely to cause injury to the leaf tissue, a significant fact in the light of the experiments reported below.

Tengwall (5), in 1924, reported some inoculations with Pestalotia from Rhododendron. He states: "Infection on living Rhododendron leaves gave positive results." Tengwall did not state his methods, but Miss Doyer (2) describes Tengwall's procedure as follows: Branches were cut off and placed in water. The leaves were then burnt in various places and on these dead areas spores were placed. The abscissed branches were then placed under a bell jar. The fungus sporulated on these injured areas and the area was increased through its action.

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Pathology.

Doyer (2) carried on rather extensive inoculation experiments with Pestalotia from Rhododendrons. Infection experiments without previous injury were always negative. Attempts made to inoculate the leaves of the variety Pink Pearl (*R. Griffithianum*) Wight, and *R. ponticum* L. through needle pricks gave negative results. Parts of leaves were burnt with a hot knife and inoculated. The organism grew in the dead areas and sporulated thereon. It also grew and sporulated on leaf tips killed with steam. No mention is made of any extension of the dead areas resulting from these inoculations. She concludes that Pestalotia is not parasitic on Rhododendrons.

During the course of a study of Rhododendron diseases, *Pestalotia macrotricha* and *P. rhododendri* have been repeately isolated from leaves of *Rhododendron ponticum* and *R. catawbiense* Hoffmgg. hybrids injured by sun scald. *P. macrotricha* has also been isolated from roots, stems, and petioles of plants previously invaded by other organisms. Observations and isolations indicated that *Pestalotia* spp. on Rhododendron were secondary organisms, invading tissue previously injured by mechanical or biological factors. The inoculations reported here on *R. ponticum*, *R. californicum* Hook., and *R. catawbiense* hybrids, made under a wide variety of conditions, were conducted in order to prove or disprove the pathogenic nature of these organisms.

Root inoculations. Inoculations on the fibrous roots have been repeatedly made both with and without injury, using spore suspensions and mycelium as inoculum. In no case has any infection taken place either on *R. ponticum*, or *R. catawbiense* hybrids grafted on *R. ponticum*, or on hybrid seedlings, with either of the organisms under discussion here.

Stem inoculations. Inoculations with *Pestalotia macrotricha* at the base of stems, near or below the soil level, have always given negative results. The majority of the inoculations in the stem have been made either on the young new growth near the top of the plant or at the growing point. This growth is soft and succulent compared to the woody growth of the previous year. Inoculations at this point, without injury, have always resulted in failure, even when a large quantity of actively growing mycelium was placed directly on the growing point of the plant kept under a bell jar. With injury, positive results have been obtained using mycelium as inoculum and inserting it directly into the wound. Using spores as inoculum, the results have been mostly negative, although some infection has resulted. Figure 1, C, D, shows a canker produced on the growing tip of a Rhododendron hybrid seedling inoculated with mycelium of *Pestalotia macrotricha*.

An attempt was made to cause infection by inserting spores and mycelium from an actively growing culture of *Pestalotia macrotricha* in the cleft made at the time of grafting. Thirty plants were inoculated at the

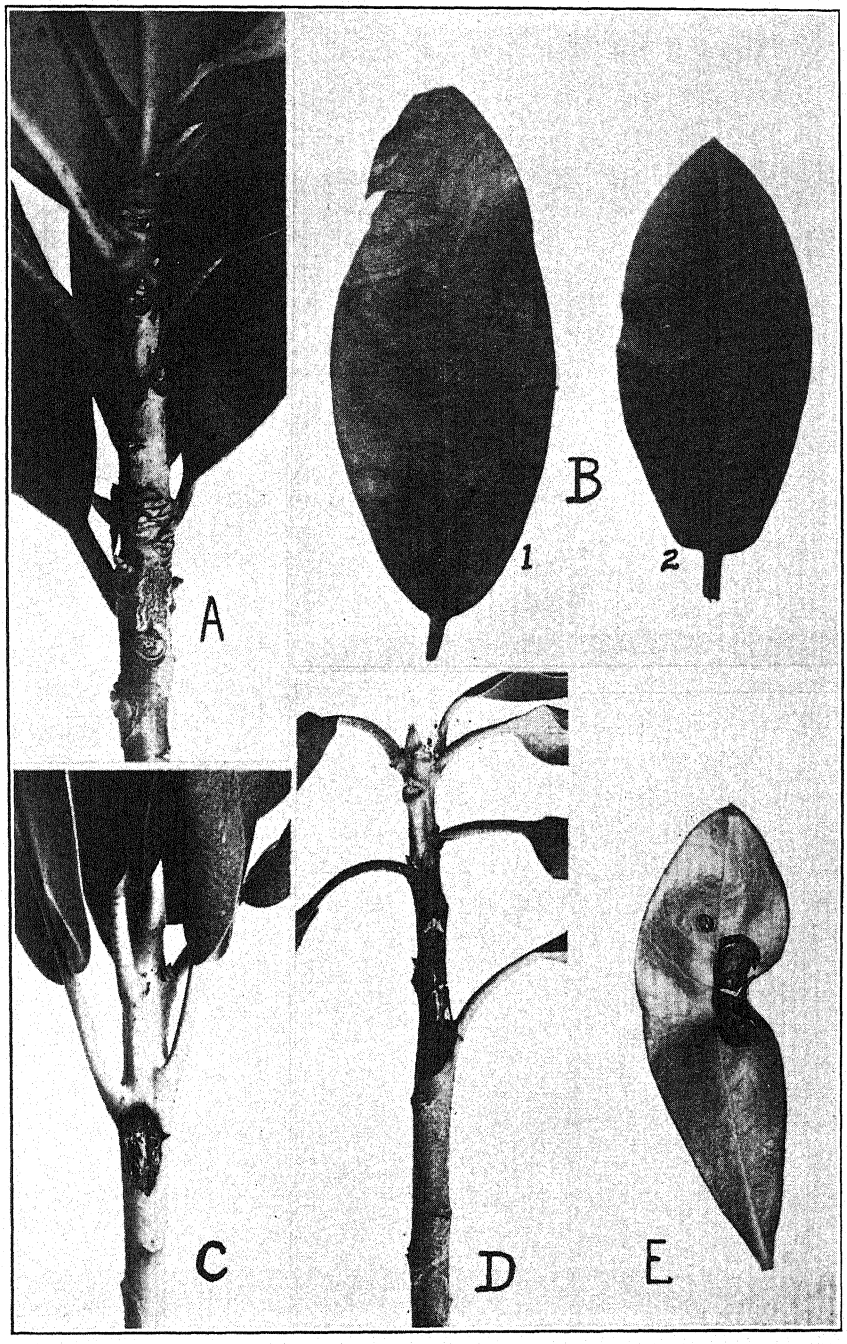


FIG. 1. A. Stem of *Rhododendron catawbiense* hybrid showing *Pestalotia macrotricha* sporulating on sun-scalded areas. B. 1. *Pestalotia macrotricha* on sun-scalded tip of *Rhododendron catawbiense* hybrid. B. 2. Natural infection of *Rhododendron catawbiense* hybrid through crack in leaf tissue. C. Lesion produced by inoculation with mycelium of *P. macrotricha* in new growth of *Rhododendron catawbiense*. D. Same as C. Twelve days after inoculation. E. *Pestalotia macrotricha* on *R. ponticum*. Inoculation by spores through needle pricks on under surface of leaf. Thirteen days after inoculation.

time of grafting by placing spores on the wound made on the *R. ponticum* stock just before the scion of *R. catawbiense* var. *album* was put in place. Thirty plants were held as controls. Callousing was perfect on all the plants and no infection was evident in any of them.

This experiment was repeated, using the same numbers and varieties of under-stock and scion wood, but substituting mycelium for spores. The results were the same. It was again repeated with a similar number of plants, using variety President Lincoln (*R. catawbiense*) as scion material, with identical results. The experiment was repeated with *Pestalotia rhododendri,* with negative results.

Inoculations in the petioles have been confined solely to petioles of mature leaves of the variety President Lincoln and to the use of spores, both with and without wounding. Inoculated plants were placed under bell jars. All attempts to cause infection at this point have failed.

Leaf inoculations. A variety of methods of inoculation of leaves has been used. The early experiments on leaves were conducted on mature leaves, using spores only as inoculum. The spores were placed in a drop of water on the upper leaf surface and the entire plant placed under a bell jar. Negative results were obtained by this method whether with or without prior injury.

Infection was also attempted on abscissed leaves placed in moist chambers. Leaves of *R. ponticum* and *R. catawbiense* var. *roseum elegans* and var. *album elegans* were first washed with mercuric chloride 1–1000 and then thoroughly rinsed in sterile distilled water under aseptic conditions. On the upper surface of these leaves, spores of *P. macrotricha* were placed in drops of water. The chambers were kept at 25° C. No cases of infection resulted, while the leaves were turgid. After ten days the leaf tissue broke down, the leaves turned black and they were invaded by the organism, which sporulated profusely.

It was found that the stomata of the species of Rhododendron used in these experiments occur only on the under surface of the leaves, varying in number from 129 to 164 per sq. mm. Accordingly, experiments were undertaken to determine whether infection could take place on the lower surface of young leaves through the stomata. Spores of *P. macrotricha* were smeared over the lower surface of several leaves of *R. ponticum* and *R. californicum.* The plants were sprayed with atomized water and placed under a bell jar. Negative results were invariably obtained, although the spores germinated and produced a fine weft of mycelium over the surface of the leaves. With injury in the form of slight leaf abrasions, infection took place, appearing after eight days on the upper surface of the leaf as a brown zonate spot.

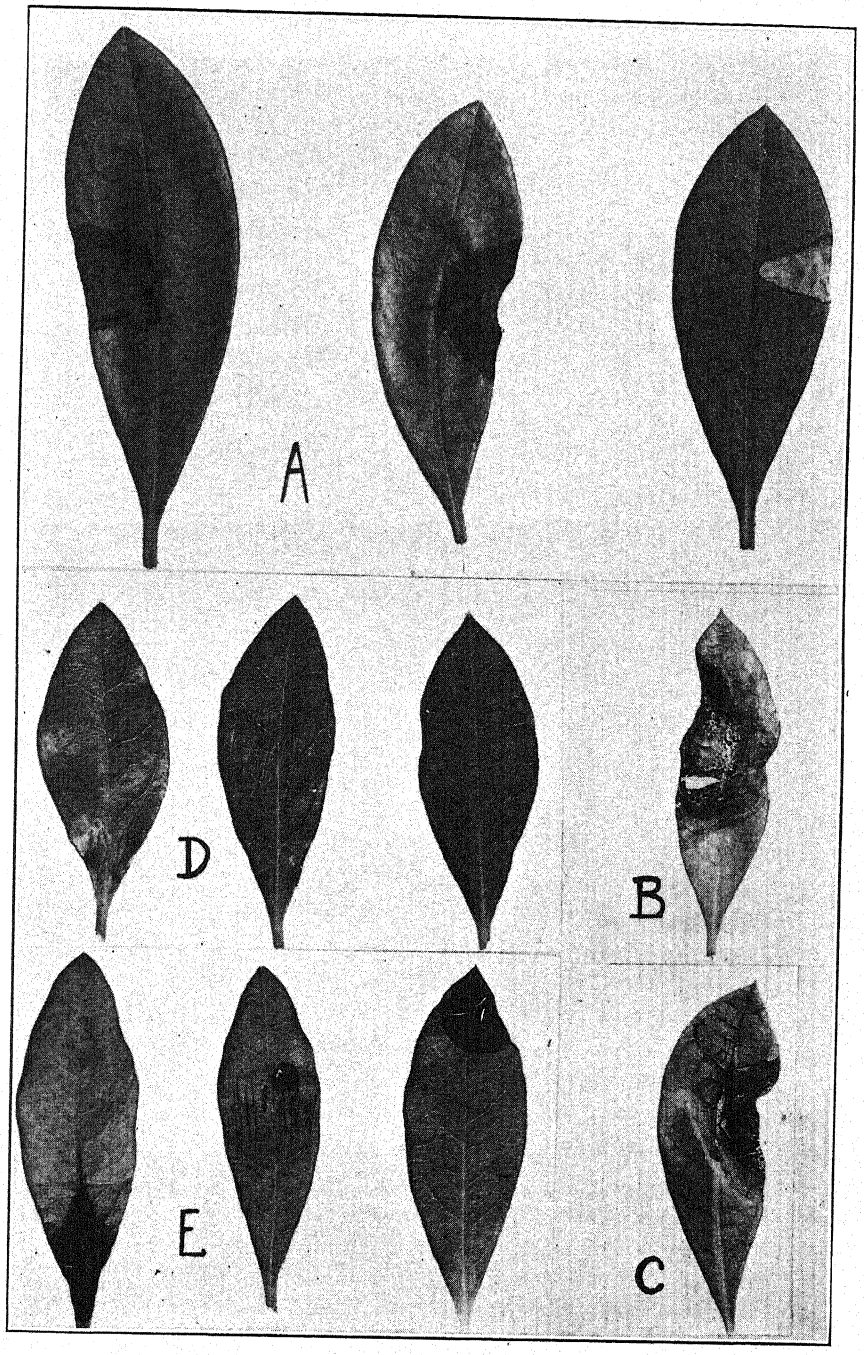


FIG. 2. A. Two leaves inoculated with *P. macrotricha* on burnt areas. Check leaf on right. Ten days after inoculation on *R. ponticum*. B–C. Same as A. Twenty days after inoculation. D. Leaves cut with sterile scalpel. Ten days after injury. E. Same as D, but inoculated with spores of *P. macrotricha*.

A further attempt was next made to inoculate young leaves of *R. ponticum* on the upper surface through cuts made with a sterile scalpel. Three young leaves on each of three one-year-old plants were cut on the upper surface with a sterile needle, one leaf cut near the base, one near the middle, and one near the tip. Spores of *P. macrotricha* were immediately smeared over the cuts and the plants atomized and placed under a bell jar. Three leaves of a fourth plant were cut in a similar manner but were not inoculated. Two days after inoculation, evidence of infection was manifest in the death of the tissue between the cuts on the youngest of the inoculated leaves. Six days after inoculation positive infection was evident on all the inoculated leaves. Brown, circular, zonate spots, typical of natural infections, appeared on these leaves. The check leaves showed no evidence of infection. Ten days after inoculation sporulation was abundant on the more advanced lesions (Fig. 2, D, E). Reisolations were successful.

Further experiments were made by placing a drop of water on the upper surface of *R. ponticum* leaves into which a hot scalpel was placed. The resulting steam killed the tissue, with the result that the latter assumed a watersoaked appearance, followed by subsequent drying and browning. Three leaves, each, of 3 one-year-old plants were thus treated. Two of the leaves on each plant were smeared with spores of *P. macrotricha*, the third leaf serving as a control.

Six days after inoculation, the burnt areas had enlarged and the advancing margin showed the characteristic concentric markings. Ten days after inoculation, sporulation had taken place in the center of the inoculated areas, and 20 days after inoculation the spots had so increased in size as to involve the larger part of the leaf. The check burns remained sterile and of the same size as when produced (Fig. 2, A, B, C). Reisolations were successful.

A final method of securing infection was as follows: With a sterile needle 100 minute punctures were made on the under surface of three *R. ponticum* leaves. Twenty-five similar punctures were made on a fourth leaf to act as controls. Spores of *P. macrotricha* were smeared over the area injured in this way. The plants were sprayed with atomized water and placed under a bell jar. The punctures were barely visible to the naked eye and were not evident on the upper surface of the leaf.

Three days after inoculation, infection was evident on two of the inoculated leaves. The area punctured was browned and invasion of the leaf tissue had extended beyond the area of injury. Thirteen days after inoculation, infection was positive on all three inoculated leaves and sporulation was abundant on the upper surface (Fig. 1, E). The punctures on the check leaf showed on the under surface as minute brown spots and were not visible on the upper surface. Reisolations were successful.

SUMMARY

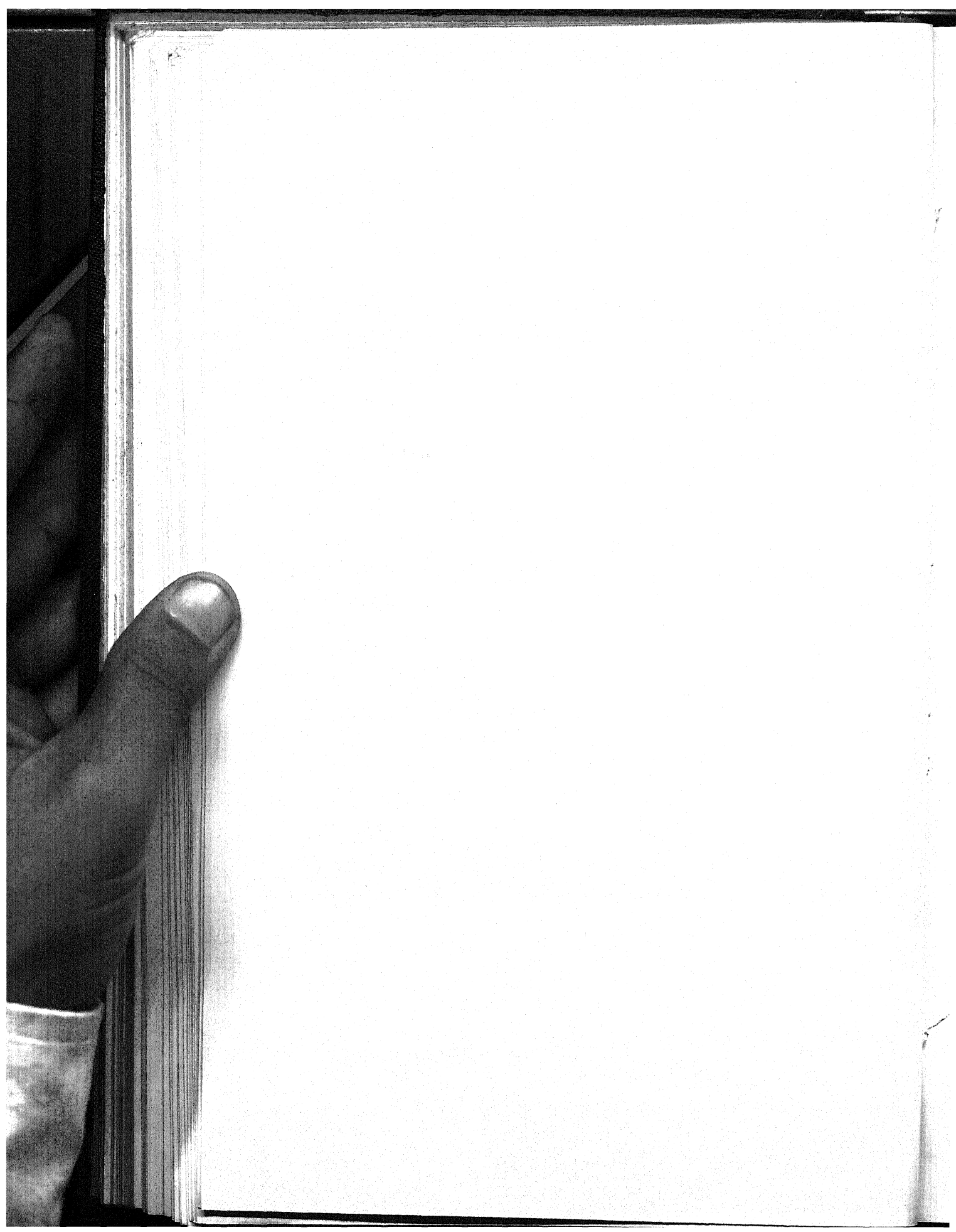
Pestalotia macrotricha and *P. rhododendri* are weak parasites on *Rhododendron* spp. In the experiments reported here they did not cause infection on roots or on old stems. *P. macrotricha,* however, caused infection on the new growth of previously injured stems; but, even then, infections were variable when spores only were used as inoculum.

On leaves, infection did not take place through the uninjured tissue or through the stomata. Positive infection obtained only through abrasions, scalded areas, and pin pricks. Once established in these injured areas, the organism invaded otherwise healthy tissue.

Various types of injury to Rhododendron leaves form excellent infection courts for these organisms. The most common of these are winter injury, sun scald, and injuries produced by thrips, lace bugs, leaf-chewing insects, and mechanical agents.

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FACTORS INFLUENCING THE MINIMUM INCUBATION PERIODS OF CURLY TOP IN THE BEET LEAF HOPPER

OLIVE SWEZY[1]

Considerable work has been carried on within recent years on the incubation period which must elapse after infection before the beet leaf hopper, *Eutettix tenellus* (Baker), is able to transmit curly top to healthy sugar beets. The problem presented by disease carriers is a complicated one that is intimately bound up, on the one hand, with the interreactions between the insect and the disease-producing organism and, on the other, with this organism and the host manifesting the disease. When this host is a plant which, to a much greater extent than is true of animals, is at the mercy of its environment, factors are introduced having little or no force in problems of animal pathology. Among such factors an important place is held by those which influence the length of the incubation period, both in the insect and in the plant.

Experimental work has shown that the effective incubation period varies within quite wide limits, from the so-called normal incubation period, or the period when at least 50 per cent of infection should be obtained, down to a single infection in as short a time as one hour. It has been found also that under normal conditions a seasonal variation occurs. A number of factors, among which may be mentioned temperature (Severin[2]), have a very direct bearing on the normal incubation periods but, when the minimum incubation periods are considered, it is evident that the same factors do not account for the percentages of infection obtained.

It has been found[3] that under normal conditions "leaf hoppers with an incubation period of one, two and three days transmitted curly leaf to 66.6, 85.2 and 88.8 per cent of the best seedlings" used. This is what we have called the normal incubation period.

In the same report it was shown that with incubation periods of one to four hours no disease was produced in 141 beets, while in 126 beets with incubation periods of four to ten hours 15 diseased beets appeared.

In later unpublished current experiments carried on by Severin slightly different results have been secured. Here an attempt was made to test periods as short as one-half hour, one hour, one-and-one-half hours and then

[1] Bureau of Entomology, United States Department of Agriculture, Berkeley, Calif.

[2] Severin, H. H. P. Minimum incubation periods of causative agent of curly leaf in beet leafhopper and sugar beet. Phytopath. 11: 424–429.

[3] Hunt, T. F. Report of Director. Calif. Col. Agr. Exp. Sta. Rept. 1922–1923: 120–132. 1923.



FIG. 1. Photomicrograph of oesophageal valve between the oesophagus and mid-intestine.



FIG. 2. Photomicrograph of an oesophageal valve between the oesophagus and mid-intestine.

every hour up to four. In these experiments one case of infection was produced with an incubation period of one half hour and one of one hour.

These short incubation periods, occurring as they do in an insect with a well-developed oesophageal valve (Figs. 1, 2) which should prevent regurgitation under normal conditions, present a problem not to be explained by the factors governing the normal incubation periods. A number of experiments have been made to test the evidence for mechanical transmission by the insect. With the 44 beet seedlings used no disease was produced. A study of the intestinal tract of the insect, however, indicates the possibility that this may occur under certain abnormal conditions.

Among the many insects examined for the oesophageal value a single individual was found in which large masses of bacteria were present in the oesophagus anterior to the valve (Fig. 3). These were in two large masses lying in the lumen of the canal. Similar masses of bacteria were to be found in the midintestine, evidently colonies that had been some time in process of formation. The sources of these masses in the oesophagus could not be determined as the valve gave no evidence of being clogged, as would be necessary to allow their egress from the midintenstine. Masses of bacteria of this type have not been found in the sugar beet. Also a comparison of the size of the masses and that of the pumping pharynx would suggest the improbability of these being intact food clumps. A few bacteria may sometimes be found in the lumen of the oesophagus. The growth of these bacteria into a colony of the size of these masses in this position could be due only to a distinctly abnormal or even pathological condition of this part of the intestinal tract. Under such conditions it might be possible for infected beet juice to be ejected from the oesophagus a short time after being taken in, as its free passage into the midintestine might be hindered. If, as seems very probable, this is the case, this condition would explain the occurrence of infection in one-half hour or one hour. It is evidently a very rare occurrence, as a single instance was found among about 250 insects examined.

The food path over which infective juice from the sugar beet must pass before it reaches the salivary glands of the insect from which the infective organism can be injected into a new plant is one that requires some time to traverse. The food must pass down the oesophagus into the midintestine, where absorption takes place. After being taken up by the blood it is carried to the salivary glands from which infective material is injected with the saliva into the plant upon which the insect feeds. The length of time necessary for the food to reach the salivary glands probably has a very direct relation to the minimum incubation period at which curly top may be transmitted to a healthy sugar beet. To determine this, the following experiments were made.

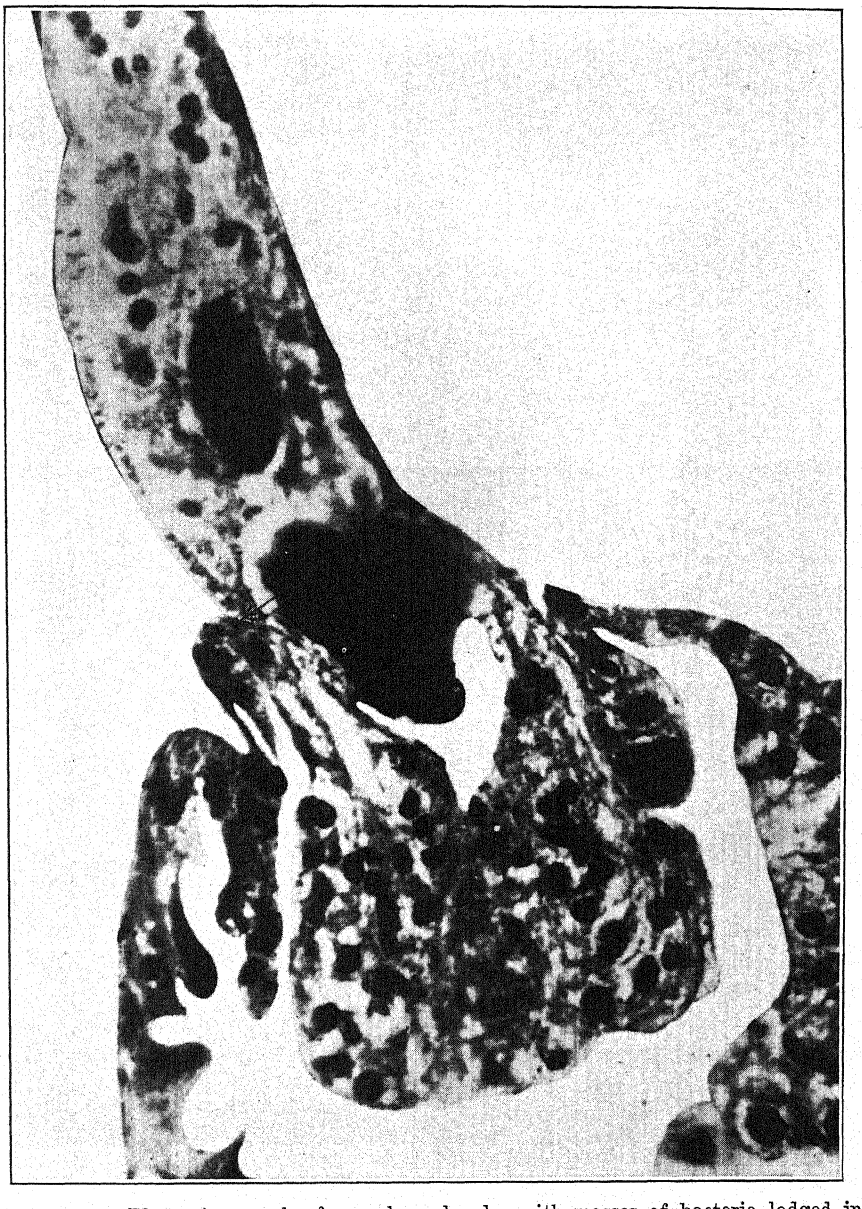


FIG. 3. Photomicrograph of oesophageal valve with masses of bacteria lodged in the oesophagus anterior to it.

Beet leaf hoppers were fasted for about twenty-four hours and at the end of that time a small dish containing the prepared food was placed in their cage. This method of feeding these insects and infecting the plants is the same as that described by Severin and Swezy.[4] The dish was filled with a 5-per-cent solution of beet sugar or sucrose in physiological-salt solution to which had been added enough stain in a 1-per-cent solution to produces a deep color. The stains used were eosin, methylene blue, and trypan blue. The last was a total failure, as it was completely oxidized as soon as it had been taken in by the insect. Of the other two, methylene blue proved the most satisfactory. This stains small granules scattered around the cell midway between the center and the periphery. The stains were prepared by dissolving the powder in a 5-per-cent sugar solution.

The first experiments were carried on under ordinary room temperatures in November and December and it was found that less than 25 per cent of the insects had fed, though some remained in the cage from three to five hours. Placing the cage under a 100-watt electric light raised this percentage somewhat, but still not enough for satisfactory work. To secure better results, the cages were placed in a box heated by two 100-watt electric lights to 100° to 115° F. Even with this, however, not more than 50 per cent of the insects would feed, those used at this time of year being the hibernating forms. The insects were taken from the cage at varying intervals and dissected under a binocular, taking out the intestinal canal and the salivary glands intact. These were placed on a clean slide in a drop of physiological-salt solution and examined under the microscope.

At room temperature it was found that within three hours insects which had fed on the prepared food showed the stain in the epithelial cells of the midintestine and the narrow portion of the malpighian tubules near the opening into the alimentary canal. In those that had been placed under a strong light the stain had been carried through the body cavity within three hours, the muscles of the wings showing a fairly heavy stain. At the end of five hours granules stained with methylene blue could be found in the salivary glands.

In these experiments no attempt was made to get the exact time of feeding, as the food was not removed from the cage until all the insects had been examined; hence the times given may in reality be somewhat less than stated, as all the insects did not begin to feed when the dish containing the sugar solution was first placed in the cage.

Since the feeding activities of these insects are largely conditioned by the temperature of their environment, and since not only the greatest percentage of infection but the minimum time of incubation are found under

[4] Severin, H. H. P., and O. Swezy. Filtration experiments on curly top of sugar beets. Phytopath. 18: 681–691. 1928.

high temperature, it was expected that the time of food absorption would be greatly lessened by high temperature. The first insects from the heated cages were examined at the end of thirty minutes, when the cells of the midintestine showed the characteristic stain. The first appearance of the stain in the salivary glands was seen at the end of one hour and fifty minutes.

In these experiments, like the first ones made, the insects were allowed to feed continuously until the last ones were taken out for examination. In later groups a number were taken from the cage and examined ten minutes later after having been fed the sugar solution. Examined at the end of fifteen minutes subsequent to such feeding, it was found that the stain had already penetrated the epithelial cells of the anterior enlarged portion of the midintestine, the staining reaction being the characteristic one found throughout these experiments. At the end of one hour stained granules could be detected in the salivary glands. No attempt was made to determine how long, after feeding, the stain could be detected in the ejected saliva. Any foreign organism or particles of stain would not necessarily have to remain in the glands the same length of time required for elaboration of the saliva from the materials absorbed from the blood.

In conducting these experiments, use was made of a 5-per-cent sugar solution, since, in previous work, this had been found the most satisfactory medium for feeding the beet leaf hopper under artificial conditions. No direct comparison can be made, of course, between this solution and the unchanged juice of the leaves of the beet upon which the insects had been fed in the experiments to determine the length of the incubation period, as no analysis of the juice of the young beet leaves has been made. With the percentages of sugar, sodium chloride, and methylene blue, it seems unlikely that the contents of the two food materials would be such as to change materially the rate of absorption when taken into the midintestine. If this is the case, then these periods represent approximately the time required under normal conditions for the absorption of food and its distribution through the body.

The question of whether we are justified in comparing methylene blue in a sugar solution with the infective organism in the beet juice is an open one. The rate of absorption would depend largely on the size of the organism unless it was able to effect entrance into the cell by its own exertions. Otherwise, it, like the stain, would be carried passively along by the movements of the surrounding liquids. The fact that ingested material may reach the salivary glands within the short interval of one hour has, however, direct bearing on the minimum incubation period of the curly-top organism in the insect.

If the organism causing curly top is passed unchanged through the body of the insect, it should be able in a large percentage of cases to transmit the disease at high temperatures one hour after feeding. This, however, is not the case, as infection at one hour is very rare. An interval of about twenty-four hours is required before at least a 50-per-cent infection is obtained, and not until the interval has lengthened to three or four days is the maximum infection secured. The time necessary for multiplication of the organism would not account for this, multiplication being necessary only to maintain a continuous infective condition in the insect. Neither can it be said that the small amount of infective material reaching the salivary glands in the first hour would preclude transmission of the disease. A single long-time-infected leaf hopper invariably gives positive results in a very large percentage of cases, while large numbers of insects, 25 to 400 or more to each plant, infected less than two hours, give negative results with rare exceptions, though it is evident that the amount of material injected into the plant is many times that produced by the single insect.

An alternative explanation is found in the fact that a change in the life cycle of the infective organism occurs in the body of the insect and this must be completed before the insect is capable of readily transmitting the disease to a healthy plant. This explanation is not incompatible with the finding of an occasional transmission of curly top within such very short intervals as one or two hours. It is possible for the infective organism to reach the salivary glands so promptly that it remains unchanged and is, therefore, ready to infect a new plant when ejected with the saliva. That this is not an habitual thing, however, is indicated by the small percentage of infection obtaining at intervals of only a few hours.

It seems probable that these two methods of internal mechanical transmission of curly top may account for the occurrence of this disease at its minimum incubation periods. One of these methods is due to an abnormal condition of the intestinal tract which hinders the free passage of food, and the other to the passage of the infective organism unchanged through the body of the insect. This, of course, does not preclude the possibility that other methods of mechanical transmission may occasionally be effective in the transmission of curly top at short intervals.

A SIMPLE METHOD OF INOCULATING THE APPLE

G. A. HUBER

During the past two years the writer has had occasion to inoculate many apples with organisms which have been isolated from the surface of normal apples to test their pathogenicity.

Granger and Horne[1] used a method of inoculation in which a cylindrical plug was removed from the apple by a cork borer. After inoculation the same plug was replaced in the cavity and sealed over with melted paraffin. The inoculated apple was treated with absolute alcohol and wrapped in sterile "greaseproof" paper.

Brooks and Cooley[2] made inoculations by forcing the spores and mycelium down into the apple by means of a platinum wire.

Previous investigations, carried out in the Plant Pathology Laboratory at the State Experiment Station, Pullman, Washington, have shown that a very high percentage of decay found in storage apples is caused by infection taking place through mechanical injuries. In order to make inoculations with pure cultures of known organisms, free from contamination, and yet as nearly as possible similar to natural injuries caused by stems, twigs, etc., the writer used the following method, which has proved more satisfactory than any other tried.

The apples are first scrubbed with a brush in running water to remove the foreign matter from the surface. Each apple is then immersed in Hg Cl_2 1–1000 for several minutes and thoroughly wiped with cotton previously soaked in the same solution and then wrung out. Punctures are made, usually three in each apple, 0.3 cm. in diameter and 0.8 cm. deep. These are made with a steel rod (Fig. 1) 0.3 cm. in diameter and provided with a collar 0.8 cm. from the end of the rod, which prevents it from sinking too deeply into the flesh of the apple. The rod is dipped into 95 per cent alcohol, then drawn through the flame of a Bunsen burner, and immediately held close to the surface of the apple, so that the flame will melt the epidermal wax around the place of puncture. If the burning alcohol is allowed to drop from the rod to the apple, a scald will develop around the puncture. When the alcohol is all burned and the wax is cooled, the rod is plunged into the apple. The inoculum is placed in the punctures and the apple is wrapped in sterile oiled wraps, which retard drying out of the punctures and prevent contamination from air and adjoining objects.

[1] Granger, K., and A. S. Horne. A method of inoculating the apple. Ann. Bot. 38: 212–215. 1924.

[2] Brooks, C., and J. S. Cooley. Temperature relations of apple rot fungi. Jour. Agr. Res. 8: 139–164. 1917.

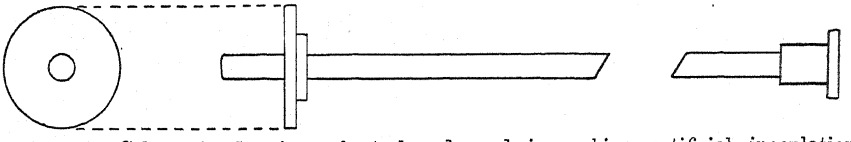


FIG. 1. Schematic drawing of steel rod used in making artificial inoculation punctures.

In order to determine the effectiveness of the melting of the wax previous to the puncturing of the epidermis of the apple, a series of puncture tests were made on apples in which the inoculating rod described above was used.

The apples to be punctured were first sprayed, by means of an atomizer, with a water suspension of spores of *Penicillium expansum* Link. The suspension was allowed to dry on the surface of the apples before puncturing them.

Fifty punctures were made without previously sterilizing the surface of the apples or melting the wax at the place of puncture; fifty punctures were made without previously sterilizing the surface of the apples, but first melting the wax at the place of puncture; fifty punctures were made after sterilizing the surface of the apples for two minutes in mercuric chloride 1–1000 and melting the wax at the place of puncture.

The data obtained in these tests are recorded in the accompanying table.

TABLE 1.—*Results obtained by melting the wax before puncturing the apples for inoculation*

Treatment	No. punctures	No. decayed	Percentage decay
Apples not sterilized; wax not melted	50	50	100
Apples not sterilized; wax melted	50	0	0
Apples sterilized; wax melted	50	0	0

The results of the tests show that the melting of the wax around the place of puncture gives heat sufficient to kill the spores which may be located on the surface or inbedded within the wax. Even though the spores are all killed around the place of puncture, it is practical to wash the apples in Hg Cl_2 1–1000 before puncturing in order to destroy the spores on the surface which may be wiped into the punctures after inoculation.

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TRANSMISSION OF CUCUMBER MOSAIC TO SPINACH[1]

ISMÉ A. HOGGAN

Of the virus diseases of the mosaic group, cucumber mosaic is regarded as having a relatively wide host range among dicotyledonous families. Not only does it affect numerous genera and species of the Cucurbitaceae and Solanaceae, but also individual species belonging to other, more or less unrelated families are known to be susceptible. It is probable that the recognized host range of this disease will become much extended in the future.

It has recently been shown[2] that cucumber mosaic is readily transmissible by the peach aphid (*Myzus persicae* Sulz.) between various members of the Solanaceae. More recently, it has been found by the writer that the potato aphid (*Macrosiphum solanifolii* Ashm.) also readily transmits this disease between tobacco and certain other solanaceous plant species. Furthermore, it has been found that cucumber mosaic is readily transmissible by these two species of aphids to spinach (*Spinacia oleracea* L.), from which host the virus is again recoverable by the same agency. The transmission of this disease to spinach is, in itself, not surprising in view of its recognized wide host range. The chief interest lies in the fact that the resulting symptoms produced on spinach bear a remarkable resemblance to those of "spinach-blight," judging from the descriptions of the latter disease as given by McClintock and Smith[3] and others. Characteristic symptoms of cucumber mosaic on spinach, as observed on young plants under greenhouse conditions, include a progressive yellowing and necrosis of the foliage, marked stunting of the plant, malformation and mottling of the young leaves, and ultimate death of the entire plant (Fig. 1). This apparent similarity of symptoms, together with the identity of the insect vectors in the two cases, is suggestive of a possible relationship between the two diseases. Unfortunately, the writer is not acquainted with spinach blight as it occurs in the field, nor has any material affected with the disease been secured. Consequently, it has not been possible to reach any definite conclusion as to the suggested identity of the two viruses in question.

[1] Cooperative investigation conducted by the Wisconsin Agricultural Experiment Station and the Office of Tobacco and Plant Nutrition, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Hoggan, I. A. The peach aphid (*Myzus persicae* Sulz.) as an agent in virus transmission. Phytopath. **19**: 109–123. 1929.

[3] McClintock, J. A., and L. B. Smith. True nature of spinach-blight and relation of insects to its transmission. Jour. Agr. Res. **14**: 1–60. 1918.

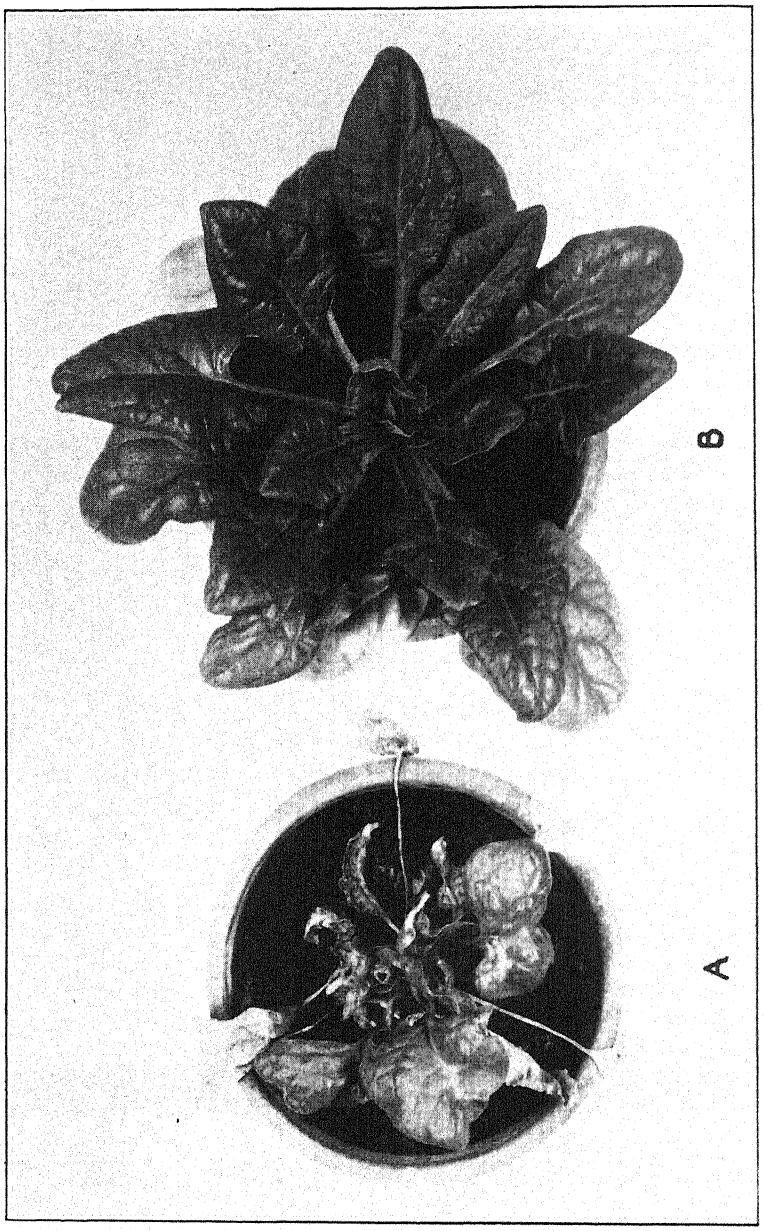
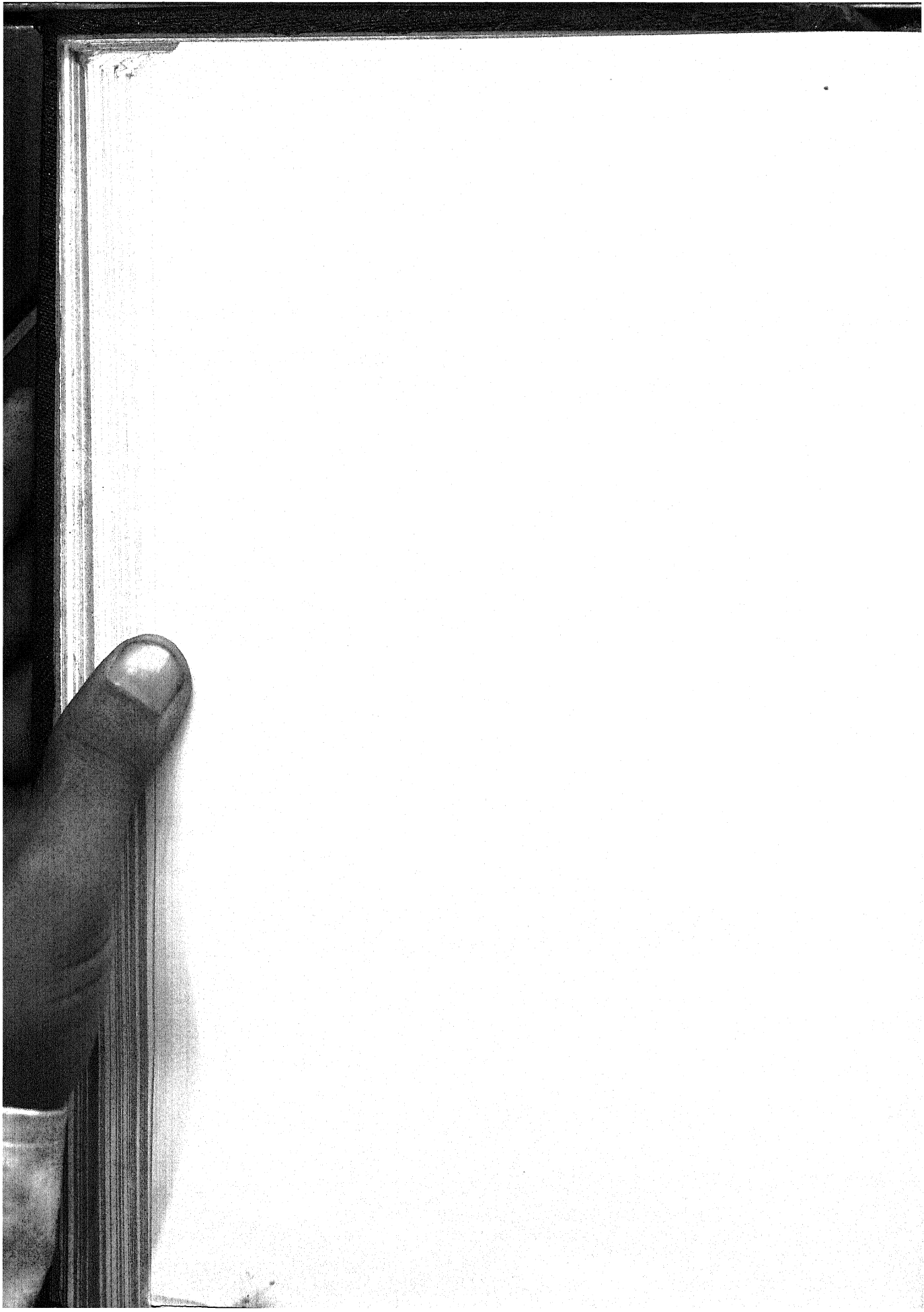


FIG. 1. A, Cucumber mosaic on spinach (variety Victoria), transmitted by *Myzus persicae*.
B, Healthy, mosaic-free plant.

Since the recognition of spinach blight as a disease of the insect-borne virus type, it has been of special interest on account of the claim made by McClintock and Smith that the virus can be transmitted directly by infective parent aphids to their progeny up to the fourth generation. If the virus of spinach blight should prove to be identical with that of cucumber mosaic, our understanding of this puzzling aspect of the virus problem would be greatly increased and simplified. However this may be, cucumber mosaic on spinach is a disease of such striking symptoms that its occurrence is believed worthy of recording. Further studies on the disease are planned, and the principal purpose of this note is to make a request for any specimens of plants known or believed to be affected with true spinach blight, in order that a more exact comparison of the two diseases may be made.

UNIVERSITY OF WISCONSIN,
MADISON, WIS.



PHYTOPATHOLOGICAL NOTES

Sclerospora macrocarpa in barley.—On June 21, 1929, J. Earl Coke gathered specimens of diseased Coast barley near Los Baños, in the San Joaquin Valley, California. The disease appeared to be *Sclerospora macrocarpa* Sacc. Specimens were sent to Wm. H. Weston, Jr., Farlow Herbarium, Cambridge, Mass., who confirmed the identification. The field, consisting of well-drained but rather heavy soil, was sown in the fall and was irrigated at least once to produce the barley crop. Rainfall in that part of California usually is insufficient to produce good grain crops. As the field was inspected after full maturity, casualties among the young seedlings could not be determined. Diseased plants were found uniformly and generally but sparsely distributed throughout the field. The attacked culms yielded spikes which produced no grain, but other culms from the same plant produced normal spikes and kernels but weak, reduced culms. The diseased spikes possessed swollen, distorted, curled awns, and twisted culms about 12 inches in length. The whole culm, including nodes and internodes, was darkened like those attacked by barley stripe (*Helminthosporium gramineum* (R.) Eriks.). The upper leaves frequently were broken into strips or ribbons by the destruction of the tissue between the veins. No conidia, but abundant oospores were observed. Further inspection of the area will be made during the next crop season.

In 1921 this disease was found on wheat in Kings County, in the same general area.—W. W. Mackie, *University of California, Berkeley, Calif.*

A Note upon the Conidial Sclerospora of Maize in South Africa.—A downy mildew of maize was observed in January, 1924, in a small plot cultivated by an Indian near Verulam, Natal. Half the plot at this time was coming into flower and showed about one per cent of diseased plants, while the young plants of the other half were about 20 per cent diseased. The affected plants exhibited the typical signs of a Sclerospora infection as described by Weston,[1] namely, chlorosis and mottling of all leaves but the lower ones, a well-marked jagged margin between the chlorotic and normal tissue of the lower leaves, upstanding position of the leaves, stunting and general weakness of the plants, and, in the older plants, fasciated tassels. Usually on visits during the daytime, there was little evidence of spore production, but this was readily induced in plants removed to the laboratory and kept wet overnight in a saturated atmosphere. Subsequently, similar disease signs were observed in plants of a sorghum cultivated alongside the affected maize.

[1] Weston, W. H., Jr. Philippine downy mildew of maize. Jour. Agr. Res. 19: 97–122. 1920.

Sclerospora was in evidence in the maize in this plot again in the seasons 1924–25, 25–26, and 26–27. Meanwhile, its sporadic occurrence was noted in many localities in the Natal Coast belt and also in the Waterberg district of the Transvaal upon maize, cultivated sorghums, and also the wild *Sorghum arundinaceum* Stapf.[2] Two cases of extensive damage to field crops of maize were observed in April, 1925, at Naboomspruit, Transvaal, and in February, 1928, at Hluhluwe, Zululand.

The direct infection of maize seedlings was readily obtained in the greenhouse by placing pieces of conidium-bearing maize leaves in proximity to the seedlings kept in a saturated atmosphere. In three experiments, 12 out of 16 plants became diseased. In similar experiments, but using diseased *Sorghum arundinaceum* as source of infection, positive infections were obtained; one out of 8 plants tested in October, 1925, one out of 10 plants in October, 1926, and 10 out of 12 plants in January, 1927. In all experiments, adjacent control plants remained free from disease.

If the foregoing experiments in transmission from *Sorghum arundinaceum* to maize may be regarded as significant, we may perhaps look to the former as an overwintering host of this fungus, since under South African conditions this grass is a weak perennial.

A more detailed account of the fungus herein referred to will be published later.—H. H. STOREY and A. P. D. MCCLEAN, *Tanganyika, Africa.*

A bacterium associated with bundle blackening in the balsam, Impatiens balsamea.—During the summer of 1924 it was observed that many of the balsam plants grown for class use in the botanical greenhouse at the University of Wisconsin had rotted at the surface of the ground. Investigation showed that not only had the stem decayed at the base but that some of the vascular bundles in each plant were blackened to the top of the stem. Attempts were made to isolate a possible causal organism by plating from bits of diseased tissue. Several organisms were isolated, including two species of Fusarium; but one bacterium was rather constantly found associated with the black bundles in the stem. The other organisms were found at the base. This bacterium was a moderate-size, actively motile rod which formed colorless colonies. Growth on nutrient agar was rapid, since at room temperature the streak on an agar slant was distinctly visible within 12 hours after inoculation and there was good growth after 24 hours.

Balsam plants were inoculated by puncturing the base of the stem with a needle which had been dipped in a pure culture of this bacterium. Posi-

[2] *Sorghum arundinaceum*, Stapf.—a weak tufted perennial under our conditions—described by Stapf as an annual in the Flora of Tropical Africa, IX, Pt. 1. p. 114; and originally included by him with *S. verticilliflorum* in *Andropogon halapensis* var. *effusus* (Flora Capensis, VII, p. 346).

tive results were secured only when the puncture was made below the surface of the ground. In this case bundle blackening occurred with great rapidity, extending to the tops of the plants, a distance of four to six inches, within 48 hours. In these small plants the black lines were visible through the transluscent stem. Since the punctures were exposed to the soil it was realized that it would be necessary to use sterilized soil in order to prove definitely that this bacterium was the cause of the disease. This was more evident since one of the check plants, which had been punctured below the surface of the ground with a sterile needle, developed blackened bundles. Time was not available for carrying out the experiment with sterilized soil, and, later, the cultures died because they had been left too long without transferring. Either the author or Dr. L. R. Jones, University of Wisconsin, would be pleased to hear from any one who has found a similar condition.—T. D. HOWE, *Table Rock, Nebr.*

A new host of sugar-cane mosaic.—While making inspections of sugar-cane fields in the vicinity of Lima for the mosaic disease during 1929, several instances of mosaic infection of "caña brava" (*Gynerium sagitattum* Beauv.) were observed. To the writer's knowledge, this grass has not been reported previously as a host of mosaic.

Infection of the wild cane has been observed only when it is growing on the borders of sugar-cane fields where mosaic is present. Both large, mature plants and young shoots arising from cut-over patches have been found affected with mosaic, the former showing both primary and secondary infection. The symptoms of the disease on the wild and cultivated hosts are identical. Although artificial inoculations have not been made to prove the identity of the disease on both hosts, field evidence supports it.

In view of the almost universal occurrence of caña brava on the banks of irrigating ditches bordering sugar-cane fields in Peru, the infection of this grass by mosaic adds another factor to be considered in controlling the disease. However, the amount of mosaic so far observed in the wild cane is relatively very small and at present it does not appear to be of great importance in spreading the disease.—E. V. ABBOTT, *Estación Experimental Agrícola de la Sociedad Nacional Agraria, Lima, Peru.*

Peteca and red blotch of lemons.[1]—Preliminary studies of peteca and red blotch (Adustiosis) of lemon fruit have been made with a view to producing the effects artificially. These efforts have yielded a few positive results. Close approximations to peteca breakdown have been secured

[1] Fawcett, H. S., and H. A. Lee. Citrus diseases and their control. 582 pp. McGraw-Hill, New York. 1926.

through hypodermic injection of small amounts of orange oil, lemon oil, and geraniol into the albedo tissue. Slight peteca-like effects also were secured by injecting methyl and ethyl alcohols. This leads one to speculate on the possible physiologic origin of the diseases, for the oils occur naturally and the geraniol and alcohols are products of anaërobic respiration. Simulations of red blotch have been effected by touching lemons with amyl formate, amyl valerate, amyl acetate, amyl propionate, and ethyl acetate, all of which esters have been found as products or by-products of anaërobic respiration in apples. Red-blotch effects were produced also by exposing the fruit to a vapor of ethyl acetate for periods of 30 minutes to 2 hours.—L. J. KLOTZ, *Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.*

Separates or reprints of the late Dr. Erwin F. Smith's article, "Fifty Years of Pathology," which appeared in the Proceedings of the International Congress of Plant Sciences held at Ithaca, are available at 80 cents per copy postpaid. The article consists of 34 pages of printed matter and 34 plates, paper cover. The plates include reproductions of about 270 pathologists. The secretary of the Congress took the responsibility of ordering 200 reprints of this paper, to be sold at a price reasonably above cost, with the understanding that any balance, after paying the cost of printing, would be made available for the general expenses of the Congress, if needed therefor, or otherwise used in some way which would obviously have been approved of by the author.

B. M. DUGGAR, *Secretary,*
International Congress of Plant Sciences.

ABSTRACTS OF PAPERS PRESENTED AT THE TWENTY-FIRST ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, DES MOINES AND AMES, IOWA, DECEMBER 28 TO JANUARY 31, 1929, INCLUSIVE

Some properties of furfural in relation to fungicides and herbicides. I. E. MELHUS.

Furfural has a low surface tension, 48 dynes, is unstable, and has a marked affinity for living or dead plant tissue. The volatility of 7 per cent furfural in water is about four times as much as in kerosene. Furfural emulsified in kerosene reduces the volatility of furfural to that of kerosene (31.85 dynes). In dilute solutions (5 per cent), furfural reduces the surface tension of water from 77 to 40 dynes and, consequently, increases the covering and penetrating powers. Furfural emulsified in kerosene (designated as FK 10–90), on the other hand, raised the surface tension less than 1 dyne.

Potato stems soaked for one hour in 7 per cent furfural in water and in kerosene gained weight, while those in furfural and FK 10–90 lost. Quack-grass rhizomes soaked in furfural and FK 10–90 for five minutes lost weight, 6.38 and 1.01 per cent, respectively. The same was true of short pieces of tomato stems. The lethal action, on green tissue, of furfural emulsified in kerosene is increased more than 10 times. Four-and-one-half times as much furfural as water is absorbed in dry apple wood, 3.7 times in green apple, and 3 times in dry red oak. Dry white cedar posts showed 25.96 per cent gain in weight in water, 10.57 in kerosene, 37.68 in furfural, and 15.33 in FK 10–90.

The waste sulphite liquor of paper mills as an adjunct to spray materials. R. H. HURT.

The waste liquor of the sulphite process of paper mills is sold under the trade names of Goulac and Glutrin. Goulac is the powdered form and Glutrin the concentrated liquor. These two forms have the same analysis, the only difference being that Glutrin contains approximately 50 per cent water. These forms are plentiful and cheap, and have been found to be valuable adjuncts to certain spray materials.

The outstanding feature of these materials is that either form makes an excellent emulsifier for home-made oil emulsions. Both forms are very soluble in water and easy to handle. The emulsions thus made are miscible in any proportion with lime-sulphur. It costs approximately one dollar to emulsify a barrel of oil with calcium caseinate and about fifteen cents with Goulac or Glutrin.

The powdered form may be substituted for calcium caseinate in the preparation of dry-mix. It makes a dry-mix which is just as wettable as one made with calcium caseinate and has better suspension qualities. The dry or liquid form when used at the rate of one pound or one quart, respectively, to 50 gallons of solution practically eliminates the chemical reaction between lime-sulphur and lead arsenate.

The Dutch elm disease. CHRISTINE BUISMAN.

In 1920 a serious disease attacked the elm tree in Holland. The symptoms are as follows: In the first part of June the leaves of the whole tree or of some branches suddenly wither and then turn brown, but they do not fall from the tree immediately and the top leaves may not fall for several weeks; the twigs curve; and in the wood conspicuous reddish brown streaks are visible. The tree may die within a year or it may live for several years. Most of the trees affected are from 15 to 40 years of age. Consequently the disease is rather rare in nurseries.

Gradually, the disease spreads through Belgium, France, and Germany; and, since 1927, it has been reported from England. There are no signs of its decreasing in the course of time.

Miss M. B. Schwartz, a Dutch phytopathologist, isolated without exception from the diseased wood a species of Graphium, which she named *G. ulmi*. Healthy wood never yields *G. ulmi*. She stated that this fungus was the cause of the disease but, as her inoculation experiments were not quite convincing, this statement has been doubted for several years. Subsequent investigators, however, could cause the disease with all its symptoms in healthy trees by inoculation with *G. ulmi*. It is likely that the spores of this fungus are spread principally by wind and enter the wood through small wounds. Means of control have not yet been found. The only thing that can be done now is to test the resistance of various species and varieties of elms by inoculation experiments and to propagate the most promising ones.

A future outlet for pathological service. R. P. WHITE.

Plant pathology as related to ornamental plant materials is the most recent field of the science to be seriously considered. The future growth of this field depends upon (1) the recognition of the increasing value of ornamentals to the great mass of our population and the importance of the industry to many of our States; (2) the ability of plant pathologists to gain the confidence of the producers and to dispel their fears concerning domestic quarantines; (3) the realization that this field touches 22 million home owners in the United States and is not limited to a small specialized group of producers; (4) the ability of department heads to gain the sympathy of the directors of our experiment stations toward the field and to receive the financial assistance necessary for research and extension activities.

Outlets for pathological service in the field are enumerated as follows: (1) positions as plant pathologist for ornamentals with many of the experiment stations located in the larger producing States; (2) positions with some of the larger nursery concerns who are able to support a plant pest expert; (3) positions on city or county park commissions who have the responsibility of maintaining the health of the ornamental plant materials used on public properties; (4) positions with "public service" corporations who sell such service to municipalities and private individuals; (5) opportunities as practicing plant doctors in many of the suburban areas where a wealth of valuable ornamental plant material has been planted, the value of which to the owner is many times the actual intrinsic value represented.

Hybridization of physiologic forms of Puccinia graminis tritici. MARGARET NEWTON, THORVALDUR JOHNSON, and A. N. BROWN.

Pustules of monosporidial origin were obtained by inoculating individual barberry plants with sporidia produced by telia of a number of single physiologic forms of *Puccinia graminis tritici*. The telia of each form had developed under controlled conditions.

The nectar on the pustules derived from the same physiologic form was intermixed ("selfing" individual forms), as was also the nectar on pustules derived from two different forms ("crossing" two forms). Only one form appeared strictly homozygous for pathogenicity; when selfed, this form alone was recovered. The other forms appeared heterozygous for pathogenicity; when selfed, each gave rise to one or more different forms, either previously known or hitherto unknown. From crosses between two forms, there arose forms differing from the parental ones, some previously known, others hitherto unknown.

From a reciprocal cross between two forms, a third form alone was recovered.

Barberry plants were inoculated with 8 forms *en masse*. Monosporidial pustules from each of the eight forms may be supposed to have developed. Some of these pustules coalesced and produced aecia. The nectar of the remaining ones was intermixed. From these inoculations were recovered several of the original forms and a number of other forms, some previously known, others hitherto unknown.

Hybridization and mutation in Puccinia graminis. E. C. STAKMAN, M. N. LEVINE, and R. U. COTTER.

Because of theoretical considerations and circumstantial evidence, it has seemed probable that different varieties and physiologic forms of *Puccinia graminis* might hybridize on barberries and that individual forms, even if selfed, might segregate if heterozygous. Craigie's discoveries furnished the necessary technique for experiments that have confirmed these probabilities. For example, from crosses between *P. graminis agrostis* and *P. graminis tritici* form 36, which apparently is relatively homozygous, several lines were obtained that are different pathogenically from both parents, and there is strong evidence that two of them never have been found in nature. Varieties and physiologic forms of *P. graminis* are dikaryotic clones which remain constant in the uredinial stage, except for mutation, but they may lose their identity in passing through the barberry because of segregation in the promycelia of the teliospores or because of the initiation of a new dikaryophase in the pycnia or aecia. The suspected rôle of barberry in the production of new forms is thus confirmed, and the facts emphasize the necessity of eradicating the bushes.

There is evidence, also, of mutation in parasitism. In a uredinial culture of *P. graminis tritici* form 1, constant pathogenically for 13 years, there suddenly appeared an apparent mutant, different pathogenically from anything the writers ever have observed. Extensive inoculations proved its constancy. It seems obvious, therefore, that new physiologic forms are arising both by hybridization and mutation. (Cooperative investigations between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

A new mosaic disease of cucumber. R. H. PORTER.

In March, 1929, a sample of mosaic on cucumbers was secured from a greenhouse at Bettendorf, Iowa. Inoculation trials produced 100 per cent infection not only in the variety White Spine, but also in selfed lines of the variety Chinese Long. Differentiation of this mosaic from the common "white pickle mosaic" is based on symptoms, period of incubation, and host range.

The symptoms of the white pickle mosaic on cucumbers are marked stunting, drooping of the terminal leaves, faint to prominent mottling, and fruit infection. Its incubation period is three to six days. Infection occurs readily on cucumbers, canteloupes, and African stock citron, but not on watermelons and the variety Chinese Long cucumber. The symptoms of the "Bettendorf mosaic" on cucumbers are rigidity of the terminal leaves with yellow blotches at first distinct, later merging together. No visible symptoms appear on the fruit. Its incubation period is 6 to 15 days. This strain gave 100 per cent infection on all cucumbers, including the variety Chinese Long. Watermelon and stock citron also are readily infected but masking may occur.

The distribution of the Bettendorf mosaic is not definitely known but, apparently, it is much less common than the white pickle mosaic.

The resistance of cucumbers to mosaic. R. H. PORTER.

Individual, mass-selected plants, as well as selfed lines of the variety Chinese Long, are highly resistant to the "white pickle mosaic" disease. Three successive inoculations into the same plants at 12-day intervals failed to produce symptoms. Repeated efforts to transfer the disease from such plants to susceptible plants failed. Occasional plants of the variety Chinese Long, which are susceptible, may mask mosaic symptoms, but this seldom occurs throughout the life of the plant. Susceptibility to mosaic is dominant, but F_1 plants of Chinese Long × White Spine are not so severely stunted as the susceptible parent. Seeds from a back cross of the F_1 × White Spine produce plants which possess the short pickling type of fruit. Part of these are susceptible and part resistant but no genetic ratios have yet been determined. When 29 plants of this back cross were exposed to two different inoculations 9 remained resistant. Three attempts to transfer mosaic to susceptible plants from these 9 were unsuccessful.

Kale yellows (Fusarium conglutinans) in California. JAMES B. KENDRICK.

A tall, large-leaf variety of kale known as "Jersey" is grown extensively in the Petaluma district, Sonoma County, California, as a green food for chickens. About 80 per cent of approximately 4,000 chicken ranchers in the district use kale as a green food for their chickens. Kale yellows, which has symptoms similar to cabbage yellows, is widely distributed in the district and is forcing many ranchers to find a substitute crop for "poultry greens." The disease has not been observed by the writer elsewhere in California.

In 1927 numerous isolations from diseased kale plants yielded pure cultures of a Fusarium which subsequent inoculations showed to be the causal agent. Comparative cultural and cross-inoculation studies of it and *F. conglutinans* showed the two to be identical.

Pathogenicity of a number of collections of the cabbage-yellows organism. L. M. BLANK.

A study has been made of the pathogenicity of a number of collections of the cabbage-yellows organism, *Fusarium conglutinans,* upon cabbage and other subspecies of *Brassica oleracea.* Pure line isolations from 11 localities in 8 States were tested, under controlled conditions of soil temperature, upon pure lines of resistant and susceptible cabbage, and upon F_2 hybrids from resistant-susceptible crosses. The homozygous resistant lines remained entirely resistant to the attack of the various collections of the organism while the homozygous susceptible strains were uniformly infected. When tested against the F_2 hybrids isolations of the fungus reacted much alike in that the segregation of healthy and diseased plants was very close to the expected 3 to 1 ratio.

No evidence of selective pathogenicity was found when the collections were tested upon other subspecies, namely, Brussels-sprouts, cauliflower, kale, and kohlrabi. Upon these hosts, as upon commercial cabbage lines, slight variations in pathogenicity were observed but were not characteristic of any one collection of the organism.

While some variation was noted in their cultural characteristics, the pathogenicity range was similar for all the collections of the organism studied.

The nature of yellows resistance in cabbage. J. C. WALKER.

Further evidence is presented to confirm earlier reports that yellows resistance in cabbage is inherited as a Mendelian dominant. Physiologic responses of homozygous resistant lines of cabbage as compared with mass-selected resistant varieties and susceptible varieties have been studied. Homozygous resistant lines remain perfectly resistant

at a constant soil temperature as high as 24° C., while Wisconsin Hollander shows relatively little resistance in constant soil temperatures above 20° C. No clean-cut yellows symptoms appear in homozygous resistant lines, even at 28° C. or above. High air temperatures increase the rate of disease development in susceptible and mass-selected resistant strains, but fail to break down the resistance of homozygous resistant lines. Severe root pruning had no influence upon the expression of resistance in the latter.

Improved homozygous resistant lines have now been established for three midseason varieties, All Head Select, Marion Market, and Globe; and for two early-market varieties, Jersey Wakefield and Golden Acre or Early Copenhagen Market. The Jersey Wakefield strain will be introduced for general use in the fall of 1930 as Jersey Queen. The Golden Acre strain will be introduced as soon as sufficient seed is multiplied.

Indexing as a control measure for the yellow-dwarf disease of onions. W. J. HENDERSON.

The symptoms of yellow dwarf are stunting, crinkling, yellowing, and drooping of the leaves. The early appearance of the disease is due to planting infected bulbs. Later it spreads to adjoining healthy plants throughout the growing season. In most of the plants that become infected early the symptoms appear currently, but in others, infected late, no signs of yellow dwarf appears until the next growing season. Eighty lots of sets (60 in each lot) selected at random at harvest time were obtained from each grower at Pleasant Valley, Iowa, in the fall of 1928. These onions were grown in the greenhouse four weeks under favorable conditions for expression of the symptoms. The percentage of disease occurring in the different tests varies from 0 to 95. Samples of the same lots of bulbs were grown in the field in the spring of 1929 and the amount of yellow dwarf determined again. The amount of yellow dwarf that developed in the greenhouse and field trials compared favorably.

It is believed that this method of indexing the bulbs in the greenhouse furnishes a reliable method of determining, in advance of the normal growing season, the amount of infection in the set stocks.

Late blight tomato rot in California. G. B. RAMSEY and ALICE A. BAILEY.

Tomato-growing sections along the coast of California have suffered severely during the past few years from epiphytotics of late blight (*Phytophthora*). Total loss of fruit in some fields has been sustained. Cars of tomatoes shipped to markets have shown 50 to 100 per cent decay. Inspection certificates covering 30 cars shipped east in 1927 showed an average of 40 per cent infection. Lesions occur on vines as well as on fruit. Infection takes place at the stem scar, the lesions appearing in about four days as light brown water-soaked blotches. Transit experiments show an average radial increase of 1.5 cm. in lesions in four days. Rate of development decreases with increase in size of lesions. Lesions held two days following four days transit showed an average radial increase of .3 cm. during these two days. There is almost no spread in transit. Tomatoes harvested from blighted fields, but showing no signs of infection when packed, developed as high as 32 per cent infection in eight days, the average diameter of these lesions being 4.3 cm. Inoculations in the stem scar and through wounds elsewhere on fruits gave 55 to 77 per cent infection. Inoculations on uninjured surfaces failed to produce infection.

The inheritance of disease resistance in the onion. G. H. RIEMAN.

Colored-bulb onions (*Allium cepa*) which exhibited a high degree of resistance to the parasitism of the onion-smudge organism (*Colletotrichum circinans*) were crossed with susceptible colorless-bulb onions. Two germs responsible for resistance to smudge

have now been discovered (1) *W*, a gene for red color; (2) W_y, a gene for yellow pigment. Two different genes for the expression of white, and therefore susceptibility, have been found to be genetically distinct (1) *I*, a dominant inhibitor of red and yellow colors; (2) *w*, a gene for white. In the F_2 and F_3 generations of crosses between red and white varieties, white bulbs have appeared which were intermediate in tests for resistance to onion smudge. The interaction of the dominant inhibitor gene *I* and the gene for red *W*, effects the production of toxic chemical entities producing intermediate resistance.

Pathogenicity of Fusarium niveum and the resistance of some watermelon hybrids. D. R. PORTER.

Seventeen cultures of *Fusarium niveum* from watermelon from widely separated localities showed differences on artificial media in rate of growth, degree of sporulation, type of pigmentation, rate of starch digestion, morphology, and ability to change the pH. Seedling rot and seedling wilt are most severe at a soil temperature of 16°–18° C., and 25°–28° C., respectively. The rate of seedling wilt was positively correlated with the degree of soil infestation. In the field there is a tendency toward positive correlation in the rate of wilting and the air temperature.

Five edible hybrids, the variety Conqueror on four commercial varieties, Nos. 30, 33, 43, 90, and 137 have been isolated. In 1928 their resistance was, respectively, 22, 35, 46, 55, and 59 per cent, while check rows planted to the variety Kleckley Sweet showed only one per cent resistance.

Two edible hybrids, Q21 and Q23, in 1928, apparently chance crosses of unknown varieties on the variety Conqueror, were 68 and 64 per cent resistant, respectively, while the checks, six commercial varieties growing in adjoining rows, showed no resistance.

The most promising of the wilt-resistant strains resulted from the isolation of a resistant genotype from the susceptible variety Kleckley Sweet. The progeny of this genotype was approximately 50 per cent resistant in 1928.

The resistance of a watermelon genotype to Fusarium niveum. J. J. WILSON and DUKE V. LAYTON.

Inbreeding and natural segregation led to further isolation of lines having increased resistance to wilt in the progeny of K-S4, a genotype isolated in 1927 from the ordinary susceptible variety, Kleckley Sweet. The progeny of 22 inbred F_2 melons showed 8.7 to 73.3 per cent resistance in the tests in 1929. Two of the F_2 melons were 8.6 and 8.7 per cent resistant, two 16.0 and 16.6 per cent, one 26 per cent, six 31 to 38 per cent, seven 40 to 46 per cent, two 50 to 57 per cent, one 62 per cent and one 73 per cent resistant. One-half of the F_2 melons were over 40 per cent resistant.

Of the 1,759 initial plants representing the entire progeny of the F_2 melons 54 per cent were alive on July 11 and 35 per cent completed the full season's growth and bore a heavy crop of high-quality melons. Only 6 per cent of the 1,731 susceptible check plants from the variety Kleckley Sweet were alive on July 11.

Control of sweet-potato stem rot. DUKE V. LAYTON.

In 1928 and 1929 experiments were conducted to determine the value of some mercury seed disinfectants when used to control stem rot of sweet potatoes by seed and slip treatment. Three disinfectants were used on eight lots of diseased and healthy seed from three varieties. The percentage of healthy plants and acre yield in bushels for the seed treatments were, respectively, as follows: mercuric chloride 67.6 and 129.2, Semesan Bel 62.9 and 141.5, Corona PD7 58.1 and 130.3, compared with 52.7 and 104.8 for the un-

treated. Two organic-mercury dip dusts were used on six lots of slips grown from diseased and healthy seed from two varieties for the control of stem rot. For the two years, Corona PD7 showed both an increase in acre yield and percentage of healthy plants, while Semesan Bel increased the percentage of healthy plants both years but it decreased the acre yield in 1928. The percentage of healthy plants and acre yield in bushels for the slip treatments were, respectively, as follows: Corona PD7 83.8 and 166.6, Semesan Bel 81.1 and 149.6, compared with 61.7 and 142.6 for the untreated.

Soil reaction as influencing Phymatotrichum root rot. WALTER N. EZEKIEL and J. J. TAUBENHAUS.

Cotton was planted in rows 12 feet long, in boxes of seven different soils, and root rot was introduced by artificial inoculation at one end of each row. The disease spread farthest in soils of pH 7.6 and 7.7; spread shorter distances in soils of pH 6.3, 6.7, and 7.1; but attacked only one inoculated plant in acid soils, pH 5.5 and 5.8. Three other inoculation experiments yielded similar results, confirming previous field-survey results and showing that root rot is destructive in alkaline soils but unimportant in acid soils.

Attempts to develop control methods based on acidification of the soil have been continued. Excessive amounts of sulphur, added to soil in small containers, acidified the soil and reduced infection. Sulphur injury occurred here as in other experiments; however, there were series in which root rot was inhibited by acidities which did not affect the host. It is impractical to acidify highly calcareous soils, so this method of control would be of value only in limited regions, and care would be necessary to prevent injury from excess acidity. The method is in the experimental stage and is not to be recommended for general use on the basis of present limited knowledge.

Overwintering and spread of Phymatotrichum root rot. J. J. TAUBENHAUS and WALTER N. EZEKIEL.

Phymatotrichum omnivorum overwinters as active mycelium on living roots of infected plants, and as sclerotia and dormant strands. Roots of cotton and weed hosts survive after frost and may live until the following fall. Root rot continues spreading along roots of plants during winter. Living, infected roots transmitted the disease readily to normal plants, while dead roots did not. The fungus developed readily from living parts but not from dead parts of infected roots. Primary infections in fields have been traced to overwintered, infected roots of cotton and *Ipomoea trifida.* In certain fields from which clean fallow had eliminated roots, infection was from sclerotia, confirming Neal's observations.

Excavation of 117 isolated plants showed that spread from these primary infections to adjoining plants was along roots and not directly through the soil. In numerous inoculation experiments, soil from around infected plants consistently failed to produce infection; though high percentages of infection resulted from inoculations with live, infected roots. In the laboratory, the fungus extended through loose soil but more extensively along the walls of the containers. As yet, there is no direct evidence that similar spread, independently of roots, occurs in the field.

Cold resistance and susceptibility in corn. J. R. HOLBERT.

In some inbred and crossbred strains of corn there is high correlation between cold resistance in the spring and in the fall. In other strains, such correlation is not very significant, due to various influencing factors.

Cold resistance, in both spring and fall, has been found to increase as productive capacity of soil increases and vice versa. However, an inherently cold-sensitive strain

will not equal an inherently cold-resistant strain in functional capacity even when grown in a highly productive soil.

Cold resistance, in both spring and fall, was markedly lower in plants growing in soil high in moisture for a few days prior to exposure to low temperatures in field refrigeration chambers than in plants, comparable in other respects, growing in soil of moderate to low moisture content.

Plants injured by cold in the seedling or juvenile stages were later in flowering and in maturity, and possessed weaker root systems, than did control plants not injured by cold in the same stages. (Cooperative investigations between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Funk Bros. Seed Company, and Illinois Agricultural Experiment Station.)

Fungicidal use of organic-mercury compounds containing furan derivatives. J. J. WILSON and C. S. REDDY.

These compounds, when used as dust disinfectants of seed corn, effectively controlled the seedling blight diseases in three years of laboratory and field experiments. Four different toxic agents were prepared by varying the amount of furfuramide, mercuric chloride, mercuric nitrate, and ammonium hydroxide and sodium hydroxide in the cannizzaro reaction.

The fungicidal action of different amounts of these toxic agents in talc was tested in the laboratory by means of the visible-root sand-culture method, and in the field in thirty-hill plots replicated ten times. Nearly disease-free, artificially and naturally Diplodia-infected, naturally Basisporium-infected, and naturally Gibberella-infected seed was used. The fungicidal action was determined by lesion count and green plant weight in the laboratory and percentage of stand and yield in the field experiments. There was a high correlation between the laboratory and field results. It was found that these toxic agents, one of which is commercially known as Sterocide, exhibited equal fungicidal properties with the now available commercial compounds being supplied for seed-corn treatment. At low temperatures these new agents were especially effective in controlling seedling blight.

Development of corn ear rot from pure-culture inoculations. B. KOEHLER.

Inoculations were made with *Diplodia zeae*, *Basisporium gallarum*, *Gibberella saubinetii*, and *Fusarium moniliforme* on open-pollinated yellow dent corn at the parts indicated: (1) on silk without disturbing husks, (2) on tip of ear, husks opened to expose ear tip, (3) beneath outer husks toward butt end of ear, (4) in shank about two inches from ear, (5) in leaf axil subtending shank, and (6) in stalk about one inch below ear bearing node. Inoculations were made at 10-day intervals from August 1, time of silking, until September 30 when maturation was well advanced. Checks were treated with sterile water in the same manner.

All organisms caused infections through silk and tip of ear, August 10 being the period when most infections were obtained at these parts. Only Diplodia or Basisporium caused infection through the shank or down the husk. Diplodia alone was able to cause ear rot when inoculations were made in leaf axil, or in stalk.

Diplodia caused a high percentage of rot in all cases throughout the period used, susceptibility being greatest during August. The other organisms produced only slight to moderate increases as compared with the control and only during more restricted periods.

Control of barley stripe (Helminthosporium gramineum). C. S. REDDY and L. C. BURNETT.

Yield data in developing seed treatments were obtained by sowing treated and nontreated seed of 20 varieties of barley in 1928 and 18 varieties in 1929. The plots were planted once in 1928, but, in 1929, 3 dates of planting treated and nontreated seed at Ames and 2 dates at Mason City, Iowa, using 2 replications each time made 10 plots each of 18 varieties.

In addition to the data on seed treatment, the occurrence of barley stripe in the plots indicated the relative resistance of the varieties. This was particularly true because all the seed used in 1929 was harvested from the 1928 plots and, therefore, had somewhat similar chances for infection.

So far, these data show that the variety Minsturdi is the most susceptible and the variety Trebi, one of the most resistant. The nine varieties more susceptible to barley stripe were benefited by seed treatment with Ceresan and with a dust consisting of 6 parts sodium bicarbonate, 6 parts sodium bisulphite, 8 parts mercury chloride, and 80 parts talc. The nine more resistant ones were not benefited. There were indications that certain varieties are susceptible to injury from these seed treatments.

Susceptibility of durum wheats and emmer to Tilletia tritici. C. S. HOLTON.

That the recent increase in bunt in durum wheats, formerly considered resistant on the basis of field observations and experiments, is due to the presence of hitherto undescribed "physiologic forms" of *Tilletia tritici* is indicated by the results of artificial inoculations made at University Farm, St. Paul, Minnesota, and summarized below.

Variety	Source of inoculum and resulting percentage of smutted heads				
	Washington	California	Manitoba	North Dakota	Minnesota
Common					
Kota	42	54	43	43	15
Preston	7	12	16	2	1
Marquis	1	3	5	0.1	0.1
Marquillo	1	1	3	0.1	0
Hope	0	0	0	0	0
Durum					
Mindum	3	5	3	51	3
Pentad	12	8	9	42	10
Emmer					
Vernal	1	1	0	8	24

The first 3 collections are approximately equal in virulence on the varieties inoculated, but that from North Dakota is especially virulent on the durums, and that from Minnesota on Vernal emmer. These and the other differences apparent from the table indicate that there are at last 3 "physiologic forms" in the 5 collections.

Inheritance of resistance to Erysiphe graminis hordei, p. f. IV. S. M. DIETZ and H. C. MURPHY.

Susceptibility to physiologic form 4 of barley mildew is dominant and due to a single pair of factors. The inoculations of the barley hybrids were under controlled greenhouse

conditions. The classification into the resistant or susceptible classes was facilitated by a clean cut segregation of the F_2 and F_3. The response of 93 pure lines of barley was determined and the following hybrids made:

Chevalier C. I. 156 (susceptible) × Goldfoil C. I. 928 (resistant).
Goldfoil C. I. 928 (resistant) × Velvet (susceptible).
Odessa C. I. 927 (susceptible) × Goldfoil C. I. 928 (resistant).
Triebsol (susceptible) × Goldfoil C. I. 928 (resistant).

Twenty-two F_1 plants produced 790 F_2 plants, 588 of which were susceptible and 202 resistant—a deviation of 4.5 plants from the expectation. In the F_3 progeny tests, 49 susceptible F_2 plants produced 414 susceptible and no resistant plants. Fifty-two susceptible F_2 segregated into 436 susceptible and 152 resistant plants in the F_3. Twenty-five resistant F_2 plants were homozygous for resistance as they produced 261 resistant F_3 plants.

Inheritance of resistance to Puccinia coronata avenae, p. f. III. S. M. DIETZ and H. C. MURPHY.

The response of the F_1, F_2, and F_3 generations to a single physiologic form of crown rust was determined under controlled greenhouse conditions. Resistance in the following crosses was dominant and due to 2 pairs of factors, one of which was an inhibitor of resistance:

Sunrise 23– resistant (SSii) × Fulghum 41– susceptible (ssII).
Sunrise 23– resistant (SSii) × Fulghum 47– susceptible (ssii).
Guyra 51– susceptible (ssii) × Sunrise 23– resistant (SSii).
Golden 84– susceptible (ssii) × Red Rustproof 11– resistant (SSii).
Algerian × Calcutta 89– resistant (SSii) × Golden 84– susceptible (ssii).

The inhibitor for resistance was expressed only in the cross, Sunrise 23 × Fulghum 41. The F_1 was susceptible, the F_2 segregated in the ratio of 3 resistant to 13 susceptible, and the F_3 progeny tests indicated distribution of the F_2 in the ratio of 7 homozygous-susceptible, 6 segregating susceptible, 2 segregating-resistant, and 1 homozygous-resistant. In the remaining crosses the F_1 was resistant, the F_2 segregated in the ratio of 3 resistant to 1 susceptible, and the F_3-progeny tests indicated the F_2 ratio to be 1 homozygous-resistant, 2 heterozygous-resistant and 1 homozygous-susceptible. These results suggest the factorial analysis indicated above when S = resistant, s = susceptible, I = inhibitor for resistance. (Cooperative investigations between Iowa Agricultural Experiment Station and Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Necrotic effect produced by wilting of susceptible varieties of wheat infected with leaf rust. K. D. DOAK.

Seedling wheat plants of Michigan Amber and Little Club susceptible to leaf rust (*Puccinia triticina* physiologic form 5) that were wilted temporarily for ½ to 2 hours at various periods after inoculation, showed immediate checking of the development of the rust. This checking was evidenced by total or partial necrosis only in the area occupied by the rust. Uredinia did not develop when plants were wilted before the time for their appearance. The tendency for partial necrosis was greater on plants wilted immediately before heading. Nonwilted plants of two susceptible varieties showed large uredinia and only slight chlorosis. Reinfection of leaves previously wilted and containing necrotic areas resulted in a development equal to that on reinfected nonwilted plants. Excess nitrogen did not alter the tendency for necrosis following wilting.

Temporary wilting was accomplished by removal and replacement of solution in sand cultures, drying and moistening of soil in pots, and by increasing transpiration rate with a

blast of dry air. All methods led to the same effect. Increase in temperature within the limit of tolerance of wilted and nonwilted plants did not modify the results.

Differentiation of symptoms and effect of leaf hopper feeding on histology of alfalfa leaves. A. A. GRANOVSKY.

Studies of alfalfa yellow top caused by *Empoasca fabae* Harris have differentiated these symptoms from other types of yellowing produced by such factors as the lack of soil moisture, high atmospheric temperature, bacterial wilt, mosaic, sudden excess soil moisture following a prolonged drought, and aphid injury. They each produce characteristic symptoms, markedly different from those caused by leaf hoppers. There is no striated discoloration of the areas between the lateral veins, which later involves the entire surface of the leaves, especially in the upper parts of the plants and in the new growth, so typical of leaf-hopper injury. There are also a shortening of internodes of the new growth, prolification, and rosetting of the new shoots in the axis of the leaves.

In leaf-hopper-injured tissue, microchemical tests revealed a greater accumulation of starch grains and sugars, especially glucose and fructose. The glucose grains were much larger than those in green tissue.

Histological studies show gradual disorganization and granulation of the plastids of affected tissue, clogging and isolation of vascular bundles by suberized or lignified layers, and complete disorganization of the phloem region of severely injured tissue. These phenomena are evidently due to enzymic secretions by leaf hoppers in a course of feeding.

Extracts of flax varieties toxic to fungi. E. S. REYNOLDS.

Unheated, sterile, water extracts made from several varieties of flax show different degrees of toxicity toward the growth of *Fusarium lini* Bolley in culture. There is some evidence of a correlation between the relative toxicity of these extracts and the degree of resistance of the flax varieties to the attack of this fungus. Stem, root, and leaf extracts likewise differ quantitatively in their toxic quality when equal quantities of dried material are used. Since the number of actively-functioning living cells in the structural parts indicated also differs similarly, the different quantitative results obtained by the present method of determining toxicity may be correlated with the amount of actively-functioning protoplasm.

Pythium root rot of strawberries in Louisiana. A. G. PLAKIDAS.

In making root-rot studies on strawberries in Louisiana, among many organisms isolated were several strains of Pythium. In a preliminary experiment, in the summer of 1928, inoculating the soil (sterilized and nonsterilized) at varying degrees of moisture content, in cans in the greenhouse, with a mixture of nine different strains of Pythium, and then bringing intact runner plants to root on the inoculated soil, root rot was produced. The effect of the pathogene on the plant is twofold: The primary roots may be vigorously attacked and become rotten, and the young plant killed in a few days; or, the attack may be slow, in which case the plant survives and continues to grow, but remains weak, chlorotic, and small in size as compared with the controls. When roots of the inoculated plants were plated, only one strain, instead of the nine used as inoculum, was reisolated. This strain has not been identified. It produces twisted, swollen hyphae (presporangia ?) in great abundance, but no spores of any kind on any of the media tried. The experiment was repeated and enlarged in the fall of 1928 with practically identical results. In this experiment, one set was inoculated with a mixture of the nine strains, as previously, and a second set with a pure culture of the single strain alone.

Root rot was produced in both cases, the disease being more severe where the single strain was used.

Fall applications of fungicides in relation to apple-scab control. G. W. KEITT.

The experiments previously reported have been continued and expanded, special attention being given to a group of arsenites (chiefly of calcium and copper) of varied composition, used individually and in various admixtures with other materials. Perithecia of *Venturia inaequalis* developed abundantly in nontreated leaves. Several of the more promising treatments reduced the number of perithecia which matured on sprayed leaves to less than one per cent of those on controls. The materials used in these treatments promise to be satisfactory with regard to effectiveness against the fungus, cheapness, and ease of application. However, the requirement of safety to the host has not been fully met. In many treatments the host injury has not been serious. In others it has been too severe for successful orchard use. The cases of severe injury from the more promising materials have usually been traceable to too early application. Amelioration of injury is being sought through delaying the treatments, dilution of materials, and modification of materials. Orchardists are cautioned against the use of these treatments until a safe and thoroughly tried method can be recommended.

Spray-oil injury in apple leaves and limbs. P. A. YOUNG.

Two to 16 per cent emulsions of petroleum oils cause chlorosis in Hibernal apple leaves. Emulsions with calcium caseinate (but not those with cresoap) produce angular mottling. Unsulphonatable parts of oils are mainly physical in their effects. A 16 per cent emulsion of a white oil was tolerated by leaves for two months, although they were visibly oily. Hypophyllous brown or purple sheens represent discolored veinlets. In severe cases, leaf margins curl upward. Toxic, aromatic, sulphonatable compounds in oils sometimes cause red or purple epiphyllous leaf spots. Large hypophyllous brown leaf spots are associated with premature defoliation. Oils enlarge wounds in leaves. Shading reduces oil injuries. Drouth increases injury from oil sprays. Early sprays may cause no symptoms until drouth occurs. Oily regions often remain green in leaves acquiring fall colors. White summer oils are fairly safe because the 0.75 to 2 per cent emulsions generally used rarely cause severe injuries. Winter oils (8 to 16 per cent emulsions) often kill buds, delay their development and cause leaf dwarfing. In sprayed apples, microtome sections stained with Oil Red O showed oil between spongy-parenchyma cells and in tracheae and vein parenchyma cells of leaves; also in tracheae, tracheoids, and pith and between phloem and collenchyma cells in stems.

Sulphur dust for the control of brown rot of peaches in storage. M. A. SMITH.

Experiments to determine the fungicidal value of Koppers dusting sulphur at different temperatures were carried on at Urbana, Illinois, during 1928 and 1929. Ripe Elberta peaches were carefully selected and divided into four lots. The first lot was brushed to remove the fuzz and was then given a uniform coating of dusting sulphur. The second lot was brushed and nondusted. The third lot was nonbrushed and nondusted. The fourth lot was nonbrushed but was given a uniform coating of dusting sulphur. After inoculation with fresh spores of *Sclerotinia fructicola*, the peaches were placed in controlled-temperature chambers ranging from 40° F. to 85° F. The amount of rot was noted at the end of 12 days.

The results showed that dusting sulphur was effective in controlling brown rot in the brushed and nonbrushed lots of peaches at the temperatures of 40, 45, and 55 degrees F.

At temperatures of 65° F. and above, no control of brown rot was obtained in any of the lots.

Brushed and nonbrushed peaches which were inoculated but to which no dusting sulphur was applied rotted readily at all the temperatures maintained.

These results show that definite fungicidal action was exerted by sulphur at a temperature as low as 40° F.

Control of yellow-leaf disease of cherries. DONALD E. BLISS.

Yellow-leaf disease (*Coccomyces hiemalis*) causes premature defoliation of cherry trees in Iowa nurseries. As a result, rapid growth and proper maturity of the wood are severely checked.

Thirteen fungicides were compared as to their effectiveness in controlling the disease on first-year budded English Morello trees. Twelve applications of fungicides were made between May 25 and August 14, 1929.

Results were based on measurements of height and caliper. One hundred and twenty-one trees sprayed with Bordeaux mixture, 4–4–50 with the addition of 1 pound of Kayso, averaged 34.01 inches tall and 7.43/16 inches caliper and exceeded similar averages of 105 untreated trees by 11.77 inches and 3.12/16 inches, respectively. Bordeaux mixture 4–4–50 produced differences of 6.125 inches height and 2.153/16 inches caliper. Koppers Thylox dust and Niagara Vitidust were as effective as Bordeaux alone. Bordeaux to which Kayso had been added was outstanding because of its ability to wet the youngest leaves and to deposit an unbroken layer of fungicide which was not easily removed by rain.

Unsatisfactory materials included: Lime-sulphur solution, 1–50 and Kayso; dusting sulphur; Koppers Ferrox and Nickel dusts; Thylox, Ferrox, and Nickel pastes (5 pounds to 50 gallons); Niagara Dry Mix and Soluble sulphurs (4 pounds to 50 gallons).

The occurrence of Gymnosporangium globosum on Pyrus malus in Iowa. DONALD E. BLISS.

Gymnosporangium globosum occurs commonly in Iowa on *Juniperus virginiana* and *Crataegus* spp. It has been found on trees of *J. virginiana* which also harbored the common cedar-apple rust, *Gymnosporangium juniperi-virginianae.*

On May 8 and 11, 1929, artificial inoculations were made on first-year budded apple trees in the nursery using galls of *G. globosum* with naturally expanded teleutosori. Sporidia which were discharged from the sori came to rest on moistened surfaces of the leaves. Distinct flecks appeared on leaves of the varieties Baldwin, Tolman Sweet, and Northwestern Greening after 13 to 19 days. Necrosis followed and no pycnidia or aecidia were formed. Noninoculated trees in the same rows showed no symptoms. Negative results were obtained from inoculation of Wealthy, Early Harvest, Ben Davis, and Benoni. Flecks of uncertain cause appeared on McIntosh, Red June, and Whitney.

Field observations on 50 varieties of apples in 1928 and on 150 varieties of apples and ornamental crabs in 1929 did not reveal aecidia of *G. globosum. Pyrus malus,* for the past two years, has not been found to serve as an alternate host of *G. globosum.*

Cane gall of black raspberry. W. M. BANFIELD.

Studies have been made of the nature and cause of the galls commonly found on black raspberry canes in Ohio, Michigan, Oregon and other States. The disease appears on fruiting canes in early summer as minute spherical galls or elongated ridges of white granular gall tissue. The galls may increase in size and number until they completely cover sections of the cane surface.

Bacteria isolated from such cane galls have differed consistently from a crown-gall-type strain, originally isolated from apple. Whereas the type strain induced large crown galls on tomato stems, the cane-gall organism induced only slight swellings. On red raspberry roots the type strain consistently induced large crown galls; the cane-gall organism, inconspicuous swellings. On black raspberry first-year canes the crown-gall-type strain induced comparatively small galls; the cane-gall bacteria, large galls similar to those from which they were isolated. From these galls, produced by inoculation, bacteria have been isolated which were typical of those inserted.

Preliminary bacteriological studies suggest that the cane-gall organism is quite different from the crown-gall-type strain. Further studies are now in progress to determine whether the cane-gall organism is a variety of *Phytomonas tumefaciens* or a separate species. (The University of Wisconsin and the Bureau of Plant Industry, United States Department of Agriculture, cooperating.)

Seasonal development of crown gall and hairy root. A. J. RIKER, E. HILDEBRAND, and G. W. KEITT.

Seasonal development studies of hairy root and crown gall of nursery apple trees were conducted during the major portion of the growing season of 1929 at Topeka, Kansas. Various meteorological records were kept throughout this period. Inoculations with pure cultures grown from single cells of the crown gall and hairy root organisms, respectively, were made at frequent intervals. The incubation periods were found to be longest in the spring or fall and shortest in the summer. The natural incidence of macroscopic hairy root was comparatively slight before the first of August, but increased rapidly thereafter and seemed to be correlated with the rapid increase in diameter of the trees. No naturally occurring crown gall was found on the trees examined. When correlated with the incubation periods following inoculations, these results suggest that under the conditions of this work the critical period for infection by the hairy root organism occurs not at the time the grafts are made but following the middle of June. However, the possibility of extended incubation periods deserves further attention. Infection was found to occur only through wounds, which ordinarily remained as open infection courts for only two days. Little if any infection was secured through uninjured callus. (The University of Wisconsin and the Bureau of Plant Industry, United States Department of Agriculture, cooperating.)

Histological studies of callus knots on apple grafts. JOHN E. SASS.

As a phase of a program of crown-gall investigations, histological studies were made of callus knots formed in the absence of a pathogen. In the apple grafts used (Wealthy on French seedlings), proliferation on the cut surfaces begins in the secondary phloem, not in the cambium. Subsequently, the secondary phloem, cambium, and primary cortex, but not the periderm, proliferate callus. The spongy cells of the respective callus masses formed by the stock and scion mingle, and together proliferate into the gap between the graft members. No elements of the woody cylinder centripetal to the cambium contribute to this process. The gap is rarely filled during the first year and no vascular bridging occurs through the filler callus. In poorly matched grafts, excessive callusing occurs and the establishing of cambial continuity between members is delayed. Subsequent cambial activity in a well-matched graft lays down a continuous and efficient cylinder of xylem and phloem. A sizable knot may form on the scion lip before the gap between members is one-third filled. The formation of this gall-like knot is conditioned, not by failure of vascular bridging through the filler callus, but by retarded establishment of cambial continuity between stock and scion.

The movement of the crown-gall organisms in the stems of tomato plants. SPAS IVANOFF and A. J. RIKER.

Studies have been made relative to the movement of *Phytomonas tumefaciens* in the stems of young tomato plants during the first four hours following inoculation. Pure-culture inoculations were supplemented by similar experiments in which each of the Gram-positive, non-motile bacteria (*P. insidiosum* and *P. michiganense*) and Burri's India ink, respectively, were mixed with crown-gall bacteria. These agents facilitated locating the less conspicuous crown-gall bacteria. Alone and in various combinations with the crown-gall organism, these agents were introduced into tomato stems through needle punctures. Dead cells of *P. tumefaciens* were also used. In histological sections prepared from this material, these agents, alone or mixed with the crown-gall bacteria, were found in three conditions; viz., as masses resembling zoogloeal strands, as clumps, and as individual bacteria or particles. After the first two minutes no significant progressive movement was observed in the 4-hour period studied. Dead crown-gall bacteria and India ink particles showed types of movement, rapidity of movement, and distributions within the tissue similar to those of living crown-gall bacteria. Various physical phenomena, such as negative pressure, capillarity, and convection currents, appear to deserve consideration in accounting for this movement.

Diseases of fruit trees in Illinois, 1922–1928. L. R. TEHON and G. L. STOUT (Presented by G. L. STOUT.)

The methods developed and used and a summmarization of the data acquired in a fruit tree-disease survey in Illinois covering the period 1922–1928 are reported upon. Distinction is made between prevalence of disease and intensity of attack. Scales for measuring the intensity of various diseases are described. From statistical indexes developed from the data, graphic presentations are made of the annual trends of diseases in their prevalence and intensity phases, and it is shown that years favorable to diseases of pomaceous fruits are generally unfavorable to diseases of drupaceous fruits. A final summary of data shows that fruit-tree diseases have had the average annual prevalence and intensities given in the following table:

Kind of tree	No. of diseases considered	Av. incidence of diseases per tree	Average intensity of disease attack		
			On fruit[a]	On twigs[b]	On leaves[c]
Apple	5	3.43	35.01	29.18	9.38
Peach	4	2.21	47.03	9.05	10.34
Plum	2	1.45	38.53	——	4.35
Cherry ...	2	1.34	15.79	——	4.30
Pear	1	.50	——	8.98	——

a, percentage of fruit infected; *b*, percentage of twigs infected; *c*, percentage of leaf area occupied by lesions. On both plum and cherry, the two leaf spots are counted one disease.

Probable identity of red and mild mosaic of black raspberries. W. H. RANKIN.

Three seasons' records of symptoms of virus diseases in a black-raspberry planting indicate that mild mosaic is an expression of red mosaic. In the Plum Farmer variety red mosaic is definitely shown in a large percentage of cases by the "black tips" of the sucker laterals. In many other individuals slightly darkened areas on the petiole of a

single leaf are the only symptoms. Between these two extremes various grades of suppression of symptoms are found. Mild mosaic on the fruiting lateral foliage was definitely correlated in 80 per cent of those showing red mosaic; only 5 per cent showing mild mosaic did not show red mosaic. The Plum Farmer population contained 2 per cent mild mosaic alone and 46 per cent red and mild combined, indicating that they are identical. Cumberland in a few cases showed symptoms of red mosaic approaching the black tip effect but more often a rosetting of the tips and occasional darkened areas on the petioles of a few leaves were the only symptoms. Mild mosaic on the fruiting laterals of Cumberland was present in 91 per cent of those showing red mosaic but 67 per cent of the plants showed mild mosaic only. That mild mosaic often represents the only symptom of red mosaic in Cumberland seems tenable.

A mosaic type in certain cases of peach yellows occurring near potatoes. GILBERT L. STOUT.

During a study of peach yellows, which, after a long period of supposed nonoccurrence in the State, has been found in Illinois since 1927, attention was attracted to a number of diseased trees with unusual foliage symptoms. The leaves of these trees were yellow-green to exceedingly pale or blanched with considerable greenness retained along the veins, which presented a marked color contrast with the rest of the leaf. The result was a striking mosaic-type appearance.

Yellows trees with this leaf coloration have had a previous history of proximity to potatoes and garden plantings.

It is suggested that more than one virus may cause peach yellows and that secondary symptoms may vary with the virus.

Slimy rot of head lettuce. J. G. BROWN.

Slimy rot of head lettuce is a field, transit, and storage disease which causes a large annual loss. It has been produced in healthy lettuce plants by inoculations, and the organisms have been reisolated. Recent studies disclose that certain conditions of the host favor infection.

Bacterial canker of tomatoes—present status of the disease. MARY K. BRYAN.

This disease has spread in recent years by means of the infected seed to the southern and western States, causing heavy losses to canners and market gardeners by reducing both quantity and quality of fruit. Often a large percentage of plants are killed before setting fruit. In other cases fruit spot has seriously reduced the amount of marketable fruit where vines had set a fair crop. Spotting of leaves and stems also occurs but appears to be of less economic importance.

Lesions are most conspicuous in the upper part of the plant; in the root a slight discoloration of the xylem is often the only noticeable symptom. As the systemic infection spreads through the cortex to the epidermis it breaks to the surface in open cankers on stems, midribs and small veins, setting free the bacteria which, under favorable weather conditions, produce secondary infections on fruits, leaves and stems.

Spotting of young fruits takes place through the numerous delicate surface hairs. Stomatal infections on leaves are at first minute, white, raised cankers; later are surrounded by a zone of dead gray tissue. If near together, large dead areas may be formed. Infection may reach the vascular system from these spots or from similar spots on stems.

Control of Bacterial canker of tomatoes. MARY K. BRYAN and O. C. BOYD.

Internal infection of tomato seed was demonstrated by planting externally disinfected seed, by cultures and by microtome sections showing bacteria in dense masses inside the seed coat just below the surface. Hence mercuric-chloride or semesan treatments give incomplete control.

Preliminary hot-water treatments of freshly cleaned and dry seed have shown that 54° C. for 1 hour retards germination slightly but does not reduce percentage of germination or sturdiness of plants. Both dry and freshly cleaned seed from infected fruit yielded *Aplanobacter michiganense* in cultures after external disinfection with mercuric chloride. Samples of the same lots of seed after treatment with hot water at 54° for 50 and 60 minutes gave no growth of the organism in cultures. Treatment for 30 and 40 minutes did not kill the organism in wet seed. As higher temperatures are injurious, this does not allow a sufficient margin for safety.

Field observations, so far, indicate that soil hold over may or may not be important. In New York there has been no recurrence of the disease for two years in fields where it was serious the previous year. It has been demonstrated, however, that the bacteria survive both summer and winter conditions in Georgia in compost from diseased plants and in fields where infected compost was used the previous year. A study of temperature and pH as influencing survival in the soil is under way.

Selection of seed from clean fields, rotation of seed beds and fields and disinfection of seed with mercuric chloride are recommended.

Further studies on the seed-corn maggot and potato black leg. J. G. LEACH.

A comparative study was made of the internal bacterial flora of the seed-corn maggot, the principal bacteria associated with black leg, and certain soil-inhabiting bacteria. It was found that certain soil saprophytes, such as *Pseudomonas fluorescens,* predominated, although pathogenic species were represented in each group. The insect should be considered as an occasional carrier of the pathogene.

Histological studies showed that bacteria of many kinds pass, apparently uninjured, through the intestinal tract of the larva. In the imago certain types of microorganisms apparently are destroyed, while certain rod-shaped species pass through uninjured. Nutritional studies indicate that bacteria, as such, are not necessary for the growth of the insect, but that they furnish available food by digesting the plant tissues. Certain germinating seedlings can be utilized in the absence of bacteria, while mature tissues can be utilized only after having been digested by microorganisms.

Survival of the potato-black-leg pathogene in the soil and some factors influencing infection. J. G. LEACH.

The black-leg pathogene survives the winter in the soil in Minnesota. The conclusion of earlier workers that this organism could not survive the winter in the soil appears not to be justified. Their conclusion was in part based on negative evidence, which, in the light of recent investigations on the methods of infection, has little or no bearing on the question. The pathogene is very resistant to low temperatures and is extremely resistant to desiccation when dried slowly in soil.

Excessive soil moisture, because it excludes oxygen, greatly inhibits the formation of wound cork by potato seed pieces. The black-leg pathogene, a facultative anaerobe, grows readily in soils too moist to permit cork formation. Under such conditions black leg can result from soil infection. Such inhibition of cork formation followed by soil infection is probably responsible for much of the development of black leg in moist soils or during very rainy seasons.

Some conditions determining potato-seed-piece decay and black leg induced by maggots. REINER BONDE.

In laboratory tests, adult flies of the seed corn maggot (*Hylemyia cilicrura*), caught in the open, did not infect potato seed pieces by direct contact. From eggs of such flies deposited in sterilized soil, maggots emerged which induced decay in potato slices in damp chambers. In seed pieces in soil, rapid decay was dependent upon shallow lesions caused by various fungi and bacteria on unhealed surfaces and upon the entrance of the maggots through such lesions. Thus, with maggots present, decay occurred in freshly cut seed pieces planted in nonsterilized soil (and attacked by fungi or bacteria), but not in suberized seed pieces planted in nonsterilized soil or in freshly cut seed pieces planted and becoming healed in sterilized soil.

In commercial potato-growing conditions in Maine, the maggots showed a similar relation to seed-piece decay, except that entrance lesions due to fungi and bacteria developed largely in storage and not in soil, even on freshly cut seed. From the inside of the puparia, *Bacillus phytophthorus* and some other potato-pathogenic bacteria have been isolated.

Similar effects were induced with seed-potato maggots (*H. trichodactyla*). Negative results were secured with the common house fly (*Musca domestica*), false crane flies (*Trichocera* sp.), a fungus gnat (*Sciara tribentata*) and various species of *Drosophila*.

The cabbage maggot as a disseminating agent of bacterial rots in the Cruciferae. REINER BONDE.

Turnips grown in the field could be grouped into three classes—(1) those with cabbage-maggot injury (*Hylemyia brassicae*) associated with a soft white rot; (2) those without maggot injury or rot; (3) those with maggot injury but without rot. The last group, however, developed the rot in storage, while turnips with no maggot injury remained healthy.

Maggots severely damaged Chinese cabbage and were found burrowing not only in the roots but up the fleshy midribs of the leaves. A rapid soft rot followed their trail. Some plants rotted before heads were formed.

Rutabagas were attacked by maggots but rapid decay was not observed in the field. However, from near the trails bacteria were isolated which were pathogenic on turnip roots, cabbage leaves, and potato slices. Such organisms could not be isolated from noninfested plants.

The wild mustard (*Brassica arvensis*) and the wild radish (*Raphanus raphanistrum*) were found generally injured at their roots by the cabbage maggot. Pathogenic bacteria often were obtained from the slightly discolored areas in the vicinity of the maggot trails but not from noninfested plants. Maggots from wild-radish roots were capable of inducing bacterial decay in slices of turnips and kohlrabi. These maggots, however, did not feed upon and infect potato slices.

Pupae of the cabbage maggot found in dry soil were surface sterilized and placed on agar plates until the flies emerged. From the inside of the puparia pathogenic bacterial cultures were secured. Similar cultures have been taken from inside mature flies caught in the field in early spring, which suggests that the pathogenic organisms overwinter inside of the pupae.

Several apparently different organisms were isolated from the hosts injured by maggots and from inside the insect pupae. These organisms have not been identified but appear from host range and symptoms to include *Bacillus carotovorus*, *B. phytophthorus* and *Bacterium campestre*.

A wilt of African daisy. B. B. MUNDKUR.

In the spring of 1929 African-daisy plants, *Dimorphotheca aurantiaca*, growing in the horticultural greenhouses of the Iowa State College, were found suffering from what appeared to be a wilt disease. Microscopic examination of the tap root and the stem showed that the xylem vessels had been invaded by fungal hyphae. Small pieces of the diseased tissue, surface sterilized and transferred to agar plates, yielded with marked purity and regularity cultures of a species of Verticillium. Seedling plants inoculated with pure cultures of the organism showed characteristic symptoms of the disease. In steam-sterilized soil infested with pure cultures of the Verticillium, plants also developed the disease. This Verticillium was compared with *V. albo-atrum* of potatoes in regard to its physiologic responses. It is similar to the potato Verticillium in respect to its growth on various media, its rate of growth, tolerance of acidity and alkalinity, and the formation of conidia.

Further progress with the control of aster wilt and yellows. L. R. JONES and REGINA S. RIKER.

Results in 1929 support those reported last year. With Fusarium wilt, selections for disease resistance again gave highly satisfactory results. Those considered stabilized in 1928 yielded like results in 1929. More recent selections approach stabilization. Promising resistant strains of all colors in type preferred by commercial florists have been secured. Trials made on "sick" soil in cooperation with John Bodger and Sons, seedsmen, Los Angeles, California, confirmed the resistance of eastern selections, and indicate the identity of the disease as it occurs in the East and the West. These trials also revealed promising new strains in commercial seeds surviving from earlier commercial culturing on sick soils. Tests are to be continued in the West where large-scale operations in the open are possible on sick soil in association with commercial seed culture.

In the prevention of yellows the use of inclosures covered with cloth of 22 x 22 threads per inch was again found effective but cloths somewhat coarser (12 x 12, 12 x 16, and 16 x 22) proved ineffective. A commercial florist found cloth-covered areas practical and profitable for cut-flower culture.

Transmission of aster yellows to the tomato. L. O. KUNKEL.

A number of unsuccessful attempts were made to transmit aster yellows to tomato plants by exposing them to yellows-carrying leaf hoppers of the species *Cicadula sexnotata*. During 1928 it was learned that a disease resembling aster yellows was prevalent on tomatoes in certain districts in Maryland. Since a number of solonaceous plants take aster yellows it was thought that the disease might be transmitted to tomatoes by grafting from closely related species. *Nicotiana rustica* was chosen for this purpose. Yellowed buds were transplanted to 12 healthy tomato plants of the Bonnie Best variety. All of the 5 plants in which the buds lived and grew developed the symptoms of aster yellows. The other 7 plants and 3 check plants remained healthy. Soon after the symptoms of yellows began to show, the *N. rustica* buds were cut out of 2 of the tomato plants. These plants did not recover but continued to show symptoms of yellows as long as they lived. It is, therefore, concluded that yellows was transmitted to the tomato. The fact that tomatoes are susceptible to this malady suggests that the disease prevalent in Maryland may be aster yellows.

Transmission of Sida mosaic by grafting. L. O. KUNKEL.

A mosaic disease common on *Sida rhombifolia* and other species of Sida in Florida and in Haiti is not transmitted by the ordinary mechanical methods. It is not carried

through the seed but is readily transmitted by budding or grafting. In symptoms and in behavior this disease closely resembles the mosaic of *Abutilon striatum* var. *thompsonii*.

Infectious chlorosis of the rose. RAY NELSON.

An infectious chlorosis of the rose has been present in the commercial rose houses of Michigan for at least four years. A recent survey of the larger houses has shown that the disease is increasing in importance, is generally present on certain varieties and that extensive losses have occurred in some instances. A loss of 9,000 plants of the Matchless variety was noted in one house. The disease usually causes a stunting of the plant, formation of midget or malformed leaflets, curling and leaf mottling of various degrees from faint mosaic speckling to extensive chlorotic areas along the veins. Flower buds on diseased plants are nearly always imperfect and salable flowers are seldom produced.

The disease has been found on a number of popular varieties of hybrid tea roses, has also been noted on Pernetianas and hybrid Pernetianas and is being widely disseminated through the shipment of diseased *Rosa Manetti* and Ragged Robin understocks. The percentage of disease observed in western plantings of these understocks has been from 10 to 100. The disease has been transmitted by budding and grafting on infected understocks but not by insects.

An infectious chlorosis of rose. R. P. WHITE.

A distinctive chlorosis of rose leaves accompanied by a general dwarfing of the entire plant, certain malformations and necroses of the leaf lamina and petioles, and frequently a root browning, was first observed in 1927 on greenhouse-grown roses. Since then it has been observed on 25 hybrid tea varieties of rose grown under glass from eight States and eastern Canada. In 1929 it was found occurring naturally on eastern outdoor-grown roses. The root browning was soon found not to be correlated with the chlorosis.

The disease has been observed occurring naturally only on hybrid tea varieties and *Rosa Manetti*. It has been experimentally transferred to *Rosa multiflora*, but attempts to transfer it to *Rosa odorata* and to *Pernetiana* and *Polyantha* types of roses have failed.

Transfer of the disease to healthy plants has been accomplished by the use of buds from diseased plants, disease symptoms appearing after one month on new growth of the originally healthy plant, in some cases far removed from the point of bud insertion. Insect-free dormant plants, when grown in insect-proof cages, have reproduced typical symptoms. Insect transfer has thus far failed.

Field control of rose diseases. R. P. WHITE.

Spraying experiments for the control of black spot of roses and certain canker diseases were conducted in two commercial nurseries on six varieties. Fourteen applications were made throughout the season, the first being made on May 15 and the final on September 24.

Excellent control of black spot was obtained with several proprietary sulphurs. Less satisfactory control was obtained with copper dusts and sprays or with concentrated lime-sulphur at the rate of 1 gallon to 50 gallons of water with 3 pounds of iron sulphate added to each 250 gallons of the spray. Control of the initial infections of brown canker and die-back due to the same disease was also obtained with many of the sulphurs but less satisfactory control was obtained with the coppers.

Data on growth responses to the various sprays used indicate that copper sprays produced a dwarfing of the plant out of proportion to the dwarfing produced by the failure to control black spot.

Two Phytophthora diseases of Rhododendron. R. P. WHITE.

Phytophthora cinnamomi has been determined as the cause of Rhododendron wilt. Most severe losses occur in *Rhododendron ponticum* and hybrid seedlings; *R. carolineanum* and *R. californicum* have been found susceptible.

The organism, a soil saprophyte, decays fibrous roots and advances up the stem in the phloem and cambium regions. Dark brown cankers are formed on the new growth where the pathogene also invades the cortex and pith and eventually the meristematic xylem elements. Infection may also take place at the soil level through growth cracks which occur during the second year's growth.

Studies on acidity and temperature-growth relations of the organism, and the effect of soil disinfectants indicate that the disease may be controlled by changing certain cultural practices and by the use of certain soil disinfectants.

Phytophthora cactorum has been found to cause a serious die-back of native and hybrid Rhododendrons of all ages. Infection has been secured by inoculations through the terminal bud and bud scales on the new growth, but not in tissue one year of age or older.

This organism has also been found to cause a leaf and twig blight of *Syringa vulgaris,* from which it is disseminated to Rhododendron. Cross inoculations have been successfully conducted.

Snow mold on turf grasses. ARNOLD S. DAHL.

Snow mold, caused by *Fusarium* spp., is common on lawns and golf courses in northern United States and Canada. The economic loss is greatest on putting greens where the disease produces grayish patches which may have a pinkish tinge. These patches are usually less than 12 inches in diameter but may run together to cover large areas. When the atmosphere is moist or snow is present and the temperature is near the freezing point, a mycelial weft covers the diseased patch. As the fungus grows under the snow, the disease is most conspicuous immediately after the snow melts. Heavy fertilization and covering the greens with straw late in the season encourage attacks of the fungus. Rye, wheat, barley, oats, fescue, creeping bent, and Kentucky blue grass have been infected in the greenhouse. A distinct difference in susceptibility has been found among the various strains of creeping bent used on putting greens. Corrosive sublimate and calomel have proven successful in controlling the disease.

Progress report on barley and wheat scab. JAMES G. DICKSON, R. G. SHANDS, P. E. HOPPE, HELEN JOHANN, and E. B. MAINS.

An epidemic of scab occurred again the past season, light in general severity, but extending westward over more area. Surveys showed severe damage in fields where wheat and barley were sown on poorly prepared corn land. Secondary spread and losses from the disease were increased materially by cutting scab-infected fields before thoroughly ripened. Control measures have developed along two lines, crop rotation and fall plowing especially for barley and the development of scab-resistant lines. (Cooperative investigations between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Wisconsin Agricultural Experiment Station, and Purdue University Agricultural Experiment Station.)

Some feeding tests with scabby barley. B. B. MUNDKUR and R. L. COCHRAN.

In the fall of 1928 many complaints were received by the Iowa Experiment Station regarding barley poisoning of hogs and poultry. This barley contained fungal hyphae in a large percentage of the grain. Perithecia were present on the surface of about 4 to 8 per cent. The fungus, on isolation, was identified as *Gibberella saubinetii.*

Hogs fed on an exclusive diet of this barley developed nausea for food and starved rather than eat it, while checks fed on clean barley were not sick and showed a slight increase in weight. After the fifth day the test animals on scabby barley began to vomit but did not develop any diarrhoea.

Mature chickens on the scabby barley showed no disease and lost no weight even when they were fed on artificially infected barley, while chickens, two weeks old, showed a loss in weight and developed a rough plumage. The birds were not on an exclusive barley diet and it was observed that they rejected a lot of the feed, picking out, as far as possible, the mash.

Guinea pigs rejected an exclusive barley diet. Fed on a half-and-half mixture of scabby barley and a mash, they lost in weight, but did not develop any disease symptoms such as was exhibited in the hogs.

Feeding scab-infected barley. B. H. ROCHE, G. BOHSTEDT, and JAMES G. DICKSON.

The farm utilization of scab-infected barley has been found economical by feeding it to cattle, sheep, and poultry. The ruminants and poultry make good gains on heavily scabbed grain with no apparent ill effect. Pigs, horses, and dogs as well as man are very sensitive to the accumulated products in the infected grains and will not tolerate low percentages of badly scabbed kernels. No method has been found to date whereby badly scabbed grain can be fed economically to pigs. Scabbed grain should be fed to cattle, sheep, or poultry on the farms rather than sold at a big discount at the elevator. (Cooperative investigations between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Wisconsin Agricultural Experiment Station.)

Report on the emetic substances in Gibberella-infected barley. ALLAN D. DICKSON, KARL P. LINK, B. H. ROCHE, and JAMES G. DICKSON.

The water extract from badly scabbed barley finely ground and extracted four to six hours was found to produce acute vomiting in pigs. The extract when freed from protein, polysaccharides and those nitrogenous substances precipitable with tannic acid is more active as an emetic for pigs than the original crude extract. The active substance or materials seemingly are associated with the fractions containing glucoside or basic nitrogen compounds. Further tests are being made with pigs and dogs in order to isolate and identify the substances. (Cooperative investigations between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Wisconsin Agricultural Experiment Station.)

Report upon scab-resistant cereal varieties. R. G. SHANDS, P. E. HOPPE, and E. B. MAINS.

Gibberella infection, scab, has played an important rôle during the past two seasons in further establishing such wheat varieties as Michigan Amber, Illinois No. 1, and Progress in the corn-winter-wheat area. There were marked differences in scab reaction in the wheat varieties tested the past few years with relatively less differences in the standard barley varieties. Testing and selecting for resistance have been difficult due to the complex relationship between the environment and the development of the diseases.

(Cooperative investigations between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Purdue University Agricultural Experiment Station, and Wisconsin Agricultural Experiment Station.)

The problem of seed transmission of the typical mosaic of tobacco. B. M. DUGGAR.

As a continuation of experiments with various substances that might be concerned in the adsorption of the virus of typical tobacco mosaic, experiments were made employing a variety of proteins and other complex substances. Although the results were varied, they were in certain instances positive; and this has led to a study of the influence of ground seeds of various types in inactivating the virus. The experiments have included seeds of diverse composition, that is, those primarily starchy and those primarily proteinaceous, representing several different families of plants.

Inactivation of the virus, which has been marked with certain proteinaceous seeds, is not a factor of the absolute protein content, but depends apparently on specific proteins or other specific material accompanying them. The ground tobacco seed is likewise capable of producing inactivation, but this is never complete at the concentrations employed. The probability of relation of transmission to adsorption and inactivation through storage proteins is pointed out.

Aphid transmission of plant viruses. ISMÉ A. HOGGAN.

The determination of the rôle played by various aphids in the transmission of specific plant viruses is of both fundamental and practical interest. That the virus of ordinary tobacco mosaic is transmitted by aphids has recently been laid open to question. Greenhouse trials have now demonstrated that *Myzus pseudosolani* and *Macrosiphum solanifolii* are capable of transmitting this virus from tomato to various solanaceous hosts, although these aphids are apparently incapable of transmitting the same virus from tobacco. On the other hand, they will readily transmit the cucumber-mosaic virus from both tobacco and tomato, as will also another species, determined as *Myzus circumflexus*. This latter aphid also appears unable to transmit the virus of ordinary tobacco mosaic from tobacco, in this respect resembling the peach aphid (*Myzus persicae*). No adequate explanation can yet be offered to account for this peculiar selective capacity of the aphids with respect to both the virus and the host plant.

It therefore appears that, although *Myzus pseudosolani* and *Macrosiphum solanifolii* may be factors in the dissemination of ordinary tobacco mosaic on tomato, none of the aphids studied is likely to be of importance in the dissemination of this disease in tobacco fields.

Cytological and bacteriological investigations of bean mosaic. RAY NELSON.

A cytological study has been made of two kinds of mosaic, mottle-type, common on pea bean, and rugose mosaic on Refugee. Free-hand, unstained section from petioles mostly have been used. These were supplemented by paraffin sections stained with iron haematoxylon and Giemsa stains. The presence of a minute organism has repeatedly been demonstrated in chloroplasts and in the cytoplasm of phloem, xylem, and parenchyma tissues of diseased plants. Invaded chloroplasts show first a small vacuolized area containing the organism. The chloroplasts are eventually destroyed and the organism is found in abundance in the plastid detritus. The organism may be localized or widely distributed in the plant. It is apparently a minute coccus usually occurring singly, in pairs, or in chains, but exhibiting considerable pleomorphism.

Using modified bacteriological methods, successful isolations have been made from very young seeds taken aseptically from immature pods and from leaf tissues from

mosaic plants grown under sterile conditions. The percentage of successful isolations apparently corresponds with the number of mosaic plants obtained from seed harvested from diseased plants. The organisms are sensitive to cultural conditions and frequent subculturing is necessary. Successful isolation and subcultures often fail without the aid of tissue cultures. Pathogenicity has not been proved. A morphologically similar organism has been isolated from highly susceptible varieties of beans showing no apparent symptoms of mosaic.

Spread of mosaic virus in tomato plants. W. A. MCCUBBIN and FLOYD F. SMITH.

A report previously presented indicated that the virus of tomato mosaic spreads through the stems at about the rate of 1–2 cm. per hour. Repetitions of this work have been carried out since that time and the results indicate that the passage of the virus is greatly delayed at temperatures somewhat lower than those presented earlier. The procedure involved rooting several branches of a tomato plant in surrounding pots so that any member of the resulting colony might be removed and grown independently, inoculation of one colony member with mosaic virus, removal of individual members thereafter at stated intervals, and subsequent observation of these to note the appearance or absence of mosaic.

When a section of the woody cylinder was removed from the stem connecting colony members the virus was apparently able to cross this point readily; and when a ring of cortex was removed at this point mosaic developed in the uninoculated shoot in several cases. It is believed that adequate precautions were taken to avoid accidental infection in these plants.

Investigations of tomato streak. S. P. DOOLITTLE and H. L. BLOOD.

Studies of tomato streak in Wisconsin show that three distinct forms of the disease exist. One of these is the familiar form produced by the combination of tomato mosaic and the juice of the potato. The other two forms, while of unknown origin, do not appear to contain the potato factor. The potato combination and one of the other forms produce a marked leaf necrosis and streaking of the stem, while the remaining form produces a severe stem streaking with occasional inconspicuous leaf necrosis.

In the expressed juice of plants affected with the potato combination the thermal death point of the virus is 65°–70° C. and its longevity *in vitro* is about 14 days. One of the other forms has a thermal death point above 80° C. and lives for at least 180 days. Similar studies with the remaining form have not been completed. Comparative inoculations using 45 species of the Solanaceae showed marked differences in the symptoms induced by each of the three forms.

Experiments with plants held at constant temperatures between 16° C. and 28° C. showed that the relative intensity of stem and leaf necrosis could be greatly altered or these symptoms entirely suppressesd by changes in temperature. The temperature reactions were distinctly different with each form of the disease.

(Cooperative investigations, Office of Horticultural Crops and Diseases, Bureau Plant of Industry, U. S. Department of Agriculture, and Wisconsin Agricultural Experiment Station.)

Mutilated seed—a contributing factor in defective stands of lima beans. W. A. WHITNEY.

In the summer of 1929 an alarming lack of stand of certain lots of California-grown lima beans was experienced by certain growers in the East. Examination of the seed revealed the presence of seed-coat and cotyledon cracking amounting to 50 to 80 per

cent. Seed lots on which no complaints were registered showed only 5 to 11 per cent similar cracking. The seed-corn maggot, diseases of fungous or bacterial origin, the use of commercial (food) stock, and old seed are factors which might add to the severity of the trouble, but no single one can be considered the cause of the lack of stand. Injury to the seed during threshing is held responsible for the mutilation and, as a result, for malformed seedlings and the generally poor stands from certain seed lots. Conditions of high temperature and low humidity in the seed-growing regions at the time of maturity and threshing result in extreme brittleness of seeds and makes them more susceptible to injury during threshing. It is suggested that more attention be paid to the speed of the threshing machine to eliminate the occurrence of a dangerously high percentage of mutilated seed.

Research on potato viruses in Montana. P. A. YOUNG.

Curly mosaic may be a genetic abnormality. Willowy tops represent one symptom of rugose mosaic. Rugose mosaic killed leaves and tops of inoculated plants. It was transmitted to potatoes separately having mild mosaic and leaf-rolling mosaic, thus indicating distinct viruses. Crinkle mosaic, leaf-rolling mosaic, mild mosaic, spindle tuber, leaf roll, and witches' broom were transmitted by core grafts. Fifty normal seed pieces, core-grafted with normal cores, produced normal plants. Mosaic in one selection of Idaho Rural potatoes apparently was not masked by temperatures of 25–30° C. Masking temperatures appear to differ for different forms of mosaic. Large, globular, intracellular bodies in rolled leaves are a symptom of leaf roll. Core grafts failed to transmit calico. Gray leaf spots in one form of calico were due to the absence of palisade cells. Tubers with witches' broom often have a normal dormancy period. Eye-bearing cores of potatoes with witches' broom grew after insertion into sugar-beet roots, producing peculiar symptoms in the beet leaves. Average reductions in yields caused by viruses: Triumph spindle tuber, 43.7 per cent; Triumph crinkle mosaic, 42.1 per cent; Gem spindle tuber, 64.3 per cent; Gem mild mosaic, 20.7 per cent; Idaho Rural crinkle mosaic, 20.4 per cent.

The viruses concerned in rugose mosaic of Irish Cobbler potatoes and the weed-host problem. W. D. VALLEAU.

Transfers from "healthy" Irish Cobbler potatoes to tobacco produce a mild disease, characterized by small necrotic rings on rubbed leaves and mild watered-silk patterns in new leaves (J. Johnson's mottle). When virulent, it produces necrotic ring-and-line patterns. (J. Johnson's ring spot—distinct from tobacco ring spot.) Transfers from rugose-mosaic Cobblers to tobacco produce "spot-necrosis" (of J. Johnson and ring spot of K. M. Smith) or predominantly vein-banding symptoms. Spot-necrosis may be separated into two distinct diseases; "healthy potato," and vein-banding (Smith's insect-transmitted disease), the latter commonly found in tobacco where potatoes have been grown. Vein-banding, from tobacco, and "healthy potato" mixed, produce spot-necrosis in tobacco and rugose mosaic in seedling potatoes; or if the healthy potato virus is virulent, a severe necrotic disease of potatoes. Vein-banding, etch, and severe etch cause mild, medium, and severe rugose symptoms, respectively, in otherwise virus-free seedling potatoes, from the tubers and second and third generation plants of which the viruses may be transferred to tobacco. Cucumber viruses also cause disease in potatoes. The viruses mentioned, and perhaps others, appear to spread from potatoes to some weeds and then to tobacco grown subsequently. As the weeds may harbor potato viruses they should be given more consideration in the potato-disease-control program.

Insect transmission of potato-virus diseases. R. W. GOSS.

For the past 4 years tests have been made of the ability of various species of insects to transmit different virus diseases of the potato. The work was conducted in the field with caged or partially caged plants of the Triumph variety. Owing to the frequent lack, or the masking, of current season symptoms, the results were obtained by indexing in the greenhouse and replanting in the field. Transmission of both spindle tuber and unmottled curly dwarf was obtained with grasshoppers, *Melanoplus* spp.; flea beetles, *Epitrix cucumeris* and *Systena elongata;* tarnished plant bugs, *Lygus pratensis;* and Colorado potato beetles, *Leptinotarsa decemlineata.* Spindle tuber was also transmitted by the leaf beetle, *Disonycha triangularis.* Less than 1 per cent of the uncaged control plants became infected with any of the virus diseases under study. A small number of tests using *Melanoplus* spp., *E. cucumeris* and *L. pratensis* with rugose mosaic, mild mosaic, and leaf roll were negative. All insects were fed on healthy plants before being used in the tests. By this means, it was found in a small number of instances, that *E. cucumeris, L. pratensis,* and *Melanoplus* spp. were carrying the virus of spindle tuber when collected.

Relation of Jimson weed to certain viruses of tomato and potato. R. W. SAMSON.

Two virus diseases of Jimson weed have been differentiated. The virus of the milder type closely resembles the virus (B of Fernow, mottle of Johnson) found in apparently healthy potatoes and can be obtained from this source. The virus of the more severe type produces more severe symptoms in tomato than that of the mild type, but resembles the latter in producing streak when inoculated into mosaic tomato plants. It has been transmitted to and recovered from 9 solanaceous species, including potato seedlings, without loss of virulence. It has not been found in uninoculated potatoes of standard varieties. It somewhat resembles the virus of Johnson's spot necrosis or rugose mosaic. Both of these Jimson-weed viruses have survived 80 days aging *in vitro* in tomato juice at 10° C. When the tomato-streak-virus complex is passed through Jimson weed the tomato-mosaic virus is not recovered. The latter produces only localized lesions in Jimson weed.

Effect of pressure in spraying potatoes with Bordeaux for control of leaf hoppers and aphis. C. F. TAYLOR and F. M. BLODGETT.

In connection with numerous spraying experiments using Bordeaux 5–5–50 in which different pressures were being compared throughout the season, quantitative records were taken of some of the diseases and insects present. The most interesting, from the standpoint of spread of virus diseases, were perhaps the opposite effects of the spray on the leaf hoppers and aphis. In respect to leaf hoppers, tip-burned leaflets per plant were counted on arbitrarily selected plants. There were most hopper-burned leaves on the unsprayed plats (average 163 per plant). At 200 pounds pressure there were 80 less and at 300 pounds 98 less than on the check. At 400 pounds pressure there was an average of 62 less than at 200 pounds. The aphis, however, varied consistently and significantly in the opposite direction. At 200 pounds pressure there were 41 more, at 300 pounds 62 more, and at 400 pounds 72 more than on the unsprayed, which averaged 20 per plant. Leaf roll plants found in one field averaged 81 more hopper-burned leaflets per plant than healthy plants. Similar differences in tip-burn between leaf roll and healthy plants appeared on sprayed and unsprayed, though the total was greater on the unsprayed.

A method of recording the distribution of copper dusts or sprays on leaves. F. M. BLODGETT and E. O. MADER.

During the past season a method of recording the distribution of copper on sprayed or dusted leaves, which may be used to supplement quantitative methods, was devised. Such a method was sought as an aid in testing the effect of different nozzles, spray-booms, pressures, and methods of dusting as they affect distribution of copper membranes on the plant. In brief the method is as follows: An iron-free paper is thoroughly wetted in a solution containing about 2 per cent potassium ferrocyanide and 5 per cent acetic acid. The paper is used while moist but without excess water. The selected sample of leaves, after wilting, is laid out on one sheet of this paper and covered with another. These are placed between pads of cotton in a strong press, such as an old-fashioned letter press. Medium pressure is applied. After about ten minutes the sheets are removed and washed in running water to remove excess chemicals. When dried these sheets form a permanent record. Areas of the leaves covered by copper are represented by brown areas on the paper, while iron, if present, will be represented by blue.

American and European leaf roll of potatoes. H. M. QUANJER and D. L. ELZE.

As phloem necrosis occurs in Dutch potato varieties suffering from leaf roll only in the veins and stalks, but in American varieties as a first-season tuber symptom (net necrosis Schultz, Folsom, Gilbert) the question of identity arises.

Apparently healthy tubers of Green Mountain, and healthy tubers of Dutch varieties, were planted alongside leaf-roll tubers of Dutch origin. Symptoms of primary leaf roll developed in the plants exposed to infection. In the Dutch varieties phloem necrosis was found only in veins and stalks, whereas in Green Mountain the new tubers also showed phloem necrosis. This was the first case of net necrosis observed by the writers.

American leaf roll has been transmitted by insects from diseased Green Mountain, Rural, and Irish Cobbler potatoes received from the U. S. A. to the Dutch varieties Duke of York, Paul Krüger (President), and Red Star in which phloem necrosis appeared only in the foliage, not in the tubers. This would seem to prove the identity of leaf roll in Europe and America.

Phloem necrosis in leaf-roll tubers of Dutch varieties can be demonstrated by allowing buds of the rose end to grow out into rootless shoots, and buds of the heel end to form roots. In the mother tuber, functioning as a stalk, secondary xylem and phloem develop, and in the primary-phloem strands necrosis can be observed.

"Pseudonetnecrosis" of the potato. H. M. QUANJER, T. H. THUNG, and D. L. ELZE.

In some strains of the variety Red Star an internal parenchyma necrosis is found as numerous brown spots inside and outside the xylem ring. It is transmitted by seed tubers to the progeny. It infects neighboring plants in the field. It has been transmitted by the aphid *Myzus persicae* from Red Star to Duke of York. No foliage symptoms are combined with it. Atanasoff, comparing it with some of the earlier figures of "netnecrosis," identified it with this American disease, and, finding it in potato plants infested with Aucuba mosaic also, considered it as a tuber symptom of this disease. Through the courtesy of Dr. Murphy the Suttons Early Regent variety was received with Aucuba mosaic, free from pseudonetnecrosis. It was later found by the present authors in several varieties which were free from Aucuba mosaic. It develops during storage, the more quickly the higher the temperature. Potato varieties differ in susceptibility—Paul Krüger (President) is very susceptible, showing the necrotic spots on the outside of the skin. A similar disease has been described by Fruwirth as "erbliche Eisenfleckigkeit" in Austrian seedling varieties. On account of its late development the transmission by seed tubers may escape attention.

Microscopic investigation of "pseudonetnecrosis" and "Kringerigheid" of the potato. L. C. P. KERLING.

The first-named disease is transmitted by seed tubers; the second occurs on certain soils only, enters during growth and manifests itself on the cut surface as concentric brown rings with the center at some point of the skin, often a lenticel, and is not transmitted by seed tubers. The rings are composed of necrotic cell groups, which resemble the necrotic cell groups found inside and outside the xylem in "pseudonetnecrosis."

In the necrotic cells the starch grains are fixed in a brown mass, consisting of a membrane, surrounding each individual grain and membranous deposits filling the spaces between the grains. These deposits are connected with the browned cell wall. After treatment with reagents they behave as suberised, lignified and pectic substances; the cell walls withstand the action of Jeffrey's fluid and Eau de Javelle better than the deposits; the deposits take the color of methylene blue, which leaves the cell walls unstained. Treatment with caustic potash decomposes the membranous deposits and shows them to be fatty products of the protoplasts.

The tissue surrounding the necrotic spots reacts as potato tissue always reacts in wounds; a wound phellem presses against the necrotic cells, it is surrounded by a wound phellogen, this in turn by a wound phelloderm. This reaction tissue is more fully developed in *Kringerigheid,* since this disease proceeds more rapidly.

Diseases of the streak type in potatoes. H. M. QUANJER and J. G. OORTWIJN BOTJES.

Core grafting has revealed the presence of different diseases of the streak type:

1. *Stipple streak* (Atanasoff). Spots and streaks in foliage and stalks; superficial necrosis in neighborhood of eyes and in eyes, causing "blindness." Showing as result of grafting with the carrier Zeeland Blue in Paul Krüger (President), Duke of York, Green Mountain and Noordeling; semilatent, resembling more or less crinkle (Murphy) and rugose mosaic (Schultz and Folsom), without tuber symptoms, in Bravo and Rose No. 4; resembling mild mosaic in Eigenheimer; latent in Zeeland Blue and two other old Dutch varieties. Red Star and a seedling of Red Star, on grafting with Zeeland Blue, behave as symptomless or nearly symptomless carriers.

2. *Stipple streak in May Queen.* Spots with strong indentations surrounding veins, scarcely any streaks, no tuber symptoms. Grafted on President it resembles streak, however without tuber symptoms.

3. *Stipple streak in Noordeling.* Milder, causing less leaf drop than stipple streak (Atanasoff). Grafted on Bravo a milder type of crinkle is produced than mentioned under 1.

4. *Stipple disease.* Spots more rounded, appearing later in the season, milder than 1, no tuber symptoms in Duke of York and Paul Krüger.

Top necrosis in the potato. J. G. OORTWIJN BOTJES and H. M. QUANJER.

Core grafting has revealed that some American and Scottish varieties and some strains of Dutch varieties are carriers of diseases causing strong necrosis chiefly in tops of plants, necrotic spots in pith and cortex, sometimes superficial necrosis of eyes, and always extended internal necrosis in tubers. Different diseases of this type exist.

1. *Yellow dwarf,* according to Barrus and Chupp, shows these symptoms in Green Mountain. In the cool climate of Holland the disease remains latent.

2. *Top necrosis latent in Green Mountain.* Grafting on Paul Krüger (President), Bravo, Red Star, Bevelander and Kerr's Pink produces top necrosis and necrosis of eyes and all other symptoms mentioned above; semilatent in Zeeland Blue, resembling mild mosaic; probably the same disease as found by Schultz (Science 62: 57).

3. *Top necrosis latent in Duke of York.* Grafting on Bravo, Red Star and Industry produces top necrosis; necrosis of eyes less evident; for the rest, complete foliage and tuber symptoms as described above; semilatent in Zeeland Blue, resembling mild mosaic.

4. *Top necrosis latent in Monocraat and some strains of Red Star.* Grafting of Monocraat on Zeeland Blue, Eigenheimer, or Duke of York via Eigenheimer reveals presence of top necrosis. No necrosis of eyes; for the rest, complete symptoms. Grafting with some strains of Red Star produces the same disease in Zeeland Blue, Paul Krüger, a seedling of Red Star, and Industrie. (Potatoes from abroad through courtesy of Dr. Murphy, Dr. Schultz and Dr. Fernow.)

Dry rot of corn caused by Diplodia frumenti and three morphologically related species. A. H. EDDINS.

Diplodia frumenti causes an ear and stalk rot of corn in Florida. The dark brown mycelium of this fungus penetrates the cob, kernels, husks, and stalks. Invaded kernels and husks of severely molded ears, as well as the pith of stalks, are blackened. Pycnidia are found on the stalks at nodes and internodes, in the interior of kernels, and embedded in stromatic masses of mycelium on the ears and husks.

Diplodia natalensis, D. tubericola, and *D. gossypina* also cause a dry rot of ears if they are artificially inoculated when in the dough stage. The symptoms of the ear rots caused by these species are similar to those caused by *D. frumenti.* The four organisms cannot be distinguished from each other in their imperfect stages, and they resemble each other in their nutritional reactions, temperature relationships, and growth on media of different hydrogen-ion concentration. They also produce the same type of rot in oranges, grapefruits, sweet potatoes, and watermelons.

Factors affecting the development of the aecial stage of Puccinia graminis. RALPH U. COTTER.

Under suitable conditions teliospores of *Puccinia graminis* may remain viable at least a year and a half. That they do not necessarily germinate during early spring rains before barberry leaves have unfolded is indicated by the fact that spores kept wet for 264 hours still were capable of causing infection. Temperatures of 12–21° C. were most favorable for the combined processes of infection and production of aecia. Infected barberries kept at 0° C. for three weeks developed pycnia when the temperature was raised to 18–20° C. Freezing killed infected barberries before it killed the rust. The minimum length of time necessary for infection by teliospores was 21 hours. On *Berberis vulgaris* infection may occur on the leaves, stems, spines, petioles, sepals, and berries. Leaves of *B. aetnensis* remained susceptible 16 days after they unfolded and those of *B. vulgaris* 12 days. Aeciospores still may be discharged 37 days after the aecia have formed and may cause infection 46 days after the appearance of the aecia. More than one physiologic form may be isolated from a single aecial cup. (Cooperative investigations between Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Minnesota Agricultural Experiment Station.)

The presence of mycelium of Peronospora schleideni in the flowers of Allium cepa. H. T. COOK.

The frequent occurrence of onion mildew on onions grown on new muck soil in New York State, the uniform distribution of affected plants in the field at the time the first signs of disease appear, and the fact that the seed crop is often attacked indicate that the fungus may be carried in the seed. Evidence supporting this was obtained in the summer of 1928 when *Peronospora schleideni* was found fruiting on the seed stalk and

flower pedicels of an onion. Sections showed that the seed stalk was permeated with mycelium which could be traced through the pedicels into the various parts of the flower. It was definitely identified as the mycelium of *Peronospora schleideni* when it was found connected with the conidiophores on the pedicels. The mycelium was found in abundance in the petals, stamens, ovary, and ovules of the flowers. Its presence in the ovules indicates that it is also present in the mature seed.

A bacterium antibiotic to Ustilago zeae. R. H. BAMBERG.

The writer isolated from corn plants inoculated but not infected with *Ustilago zeae* in the field at St. Paul, Minnesota, a bacterium that prevents normal infection and also destroys colonies of the smut fungus growing on artificial media. When corn was inoculated with certain lines of *U. zeae,* approximately 70 per cent of the plants became normally infected, but infection was almost entirely prevented when the bacterium was added to the inoculum, incipient or abortive galls only being formed on a few plants. The bacterium also may destroy smut galls after they have been formed.

Colonies of *U. zeae* on artificial media were almost completely destroyed 10 to 14 days after inoculation with the bacterium, which apparently caused the disintegration of the sporidia. The bacterium also inhibits the development of colonies of *U. avenae, U. levis, Sorosporium reilianum,* and *Tilletia tritici.* The presence of the organism itself seems necessary to produce the deleterious effect, filtrates so far having proved innocuous. The writer tried many other bacteria but they had no effect.

The relationship of Bacterium medicaginis phaseolicola and Bacterium puerariae. FLORENCE HEDGES.

Cross inoculations with pure cultures of *Bacterium medicaginis phaseolicola* and *Bact. puerariae* have resulted in the production of typical kudzu halo spot and bean halo blight, confirming the suspicion of the writer that we are here dealing with one organism instead of two.

Successful cross inoculations have likewise been obtained by Boyd, who, however, did not use pure cultures but an inoculum obtained by soaking diseased leaves in water for 2 to 5 hours.

The name *Bact. medicaginis phaseolicola* (*Phytomonas medicaginis phaseolicola*) was published in PHYTOPATHOLOGY, December, 1926; that of *Bact. puerariae* in PHYTOPATHOLOGY, January, 1927; hence the latter becomes a *nomen nudum.*

Before the publication of the second paper on *Bact. puerariae* the writer had discovered bean halo blight in the west and, because of the striking similarity in the symptoms of the two diseases and the organisms associated therewith, she suspected a common cause. At this juncture she was by no means sure that the western bean disease was identical with the one described by Burkholder in New York as due to *Bact. medicaginis phaseolicola,* since his description gave a considerably different picture from that exhibited by the western fields. Hence the name *Bact. puerariae* was allowed to stand pending further investigations.

In the meantime, recent experimental work and more extensive field surveys, including observations on Burkholder's bean plots at the Cornell Experiment Station, have left no doubt that the halo blight of bean in the west, middle west, and south and kudzu halo spot are identical with the New York disease of bean due to *Bact. medicaginis phaseolicola* Burkholder.

An albino strain of Aplanobacter michiganense. MARY K. BRYAN.

A pure-white strain of *Aplanobacter michiganense* has been separated from three different isolations at various times. The loss of color is permanent on culture media. Inoculations on tomatoes with this strain have produced typically diseased plants from which the white organism has been reisolated. This strain, which is Gram-positive and non-motile, agrees with *A. michiganense* in all of the cultural tests made.

Effect of ultra-violet radiation upon sporulation in Macrosporium and Fusarium. G. B. RAMSEY and ALICE A. BAILEY.

Definite stimulation of spore production occurs in cultures of *Macrosporium tomato,* and of *Fusarium cepae* on exposure to ultra-violet radiation produced by a quartz-mercury arc. Increased sporulation appears to be a result of stimulation rather than an indirect result of inhibitory action. Greatest sporulation occurs when filters are used the lower limits of transmission of which are between 2535 and 2800 Ångstrom units. Lethal effects appear with shorter wave-lengths. Slight stimulation results when filters transmitting no lower than 3120 Å. U. are used, but none with those transmitting only to 3334 Å. U. Great increase of sporulation occurs with irradiations of 15 to 30 minutes. Stimulation of spore production is not due to changes of temperature nor to change of the culture medium as a result of irradiation. Long exposure to intense direct sunlight through filters transmitting no lower than 3120 Å. U. induces abundant sporulation. However, the experiments with the mercury arc tend to show that the difference in amount of sporulation under Vitaglass (transmitting to 2650 Å. U.) and under filters transmitting as low as 3020 to 3120 Å. U. is not due solely to a difference in intensity in this region but to a difference in the total energy transmitted.

Standardization technique in certain virus studies. B. M. DUGGAR.

Crude juice from mosaic-diseased or healthy tobacco leaves pressed out or filtered through cotton or filter-paper contains a variety of particles which interfere with many operations. Adsorption studies with the virus of typical tobacco mosaic have suggested proximate methods of purification by removal of these grosser particles. A diatomaceous trade preparation called supercelite has proved exceptionally valuable in purification technique; and, in connection with fractional dilution, the use of this substance has become a standard procedure.

Thermal inactivation tests, rendered increasingly more necessary owing to the use of temperature as a criterion in distinguishing certain forms of mosaics, have been standardized by the use of a more adequate direct immersion method and glass capillaries.

Stomatal infection with the virus of typical tobacco mosaic. BURT JOHNSON and B. M. DUGGAR.

Extensive series of experiments have been made with the view of determining the conditions limiting stomatal infection in tobacco mosaic. The experiments were conducted both in the greenhouse and in an insect-proof tent in the field. In order to secure data on any possible correlations between the diurnal movements of the stomata of the host and the production of disease, applications of the virus were made by means of an atomizer, lots of 10 plants being sprayed hourly throughout the day in two series. Records of general weather conditions, temperature, and humidity were taken. At the time of each spraying strips from both the upper and the lower epidermis were removed and placed in absolute alcohol. Subsequent study of the stomatal conditions indicated that all stomata are never completely closed, and that the stomatal diurnal movements are not

correlated with the environmental conditions within the limits employed. Moreover, the percentage of disease production is not correlated with these environmental changes nor in any way related to the time of day at which sprayings were made. An outstanding feature of these results is the high rate of infection through the stomata as entrance channels, presumably. The incidence of disease averages about 80 per cent.

The effect of some mosaic diseases on the cell structure and the chloroplasts. MELVILLE T. COOK.

Studies on the sugar cane and tobacco mosaics have been continued, together with the mosaic diseases of tomatoes, cowpeas, *Abutilon hirtum*, and canna. Tissues from chlorotic and green areas of diseased leaves were compared with tissues from normal leaves of approximately the same age. These studies confirm the statements made by many writers that the chlorotic areas are thinner than the green areas or than normal green leaves of the same age, the palisade is undeveloped, the mesophyll is reduced, and the cells compact. These modifications vary with the severity of the attack and the development of the tissues at time of attack. It is due to inhibition.

The chloroplasts in the chlorotic areas are reduced in size and number in the very young leaves but increase in size and number with age and exposure to sunlight until the patterns almost or completely disappear. This can be traced in sections made from a series of leaves beginning with the very youngest in which it is possible to detect the pattern. This appears to be due to inhibition and not to disintegration as stated by many writers. In the case of the *Abutilon hirtum* there is little or no tendency for the chloroplasts to become normal and the chlorotic areas do not become green.

Physiologic forms of barley mildew, Erysiphe graminis hordei. E. B. MAINS and S. M. DIETZ.

It has been shown by Marchal, Reed, and Salmon that the powdery mildew of barley is restricted to a few closely related species of Hordeum such as *H. vulgare*, *H. intermedium*, *H. distichon*, and *H. difeciens*. These studies have resulted in discovering a number of very resistant varieties in these species of Hordeum. Using such varieties, five physiologic forms of the mildew have been differentiated. Thus Nepal C. I. 595 is resistant (type 1–2) to physiologic form 1 and susceptible to the other four physiologic forms. Peruvian C. I. 935 is resistant (0–1) to physiologic forms 1, 2, and 4 and susceptible (4) to physiologic forms 3 and 5. Black Hulless C. I. 666 is resistant (1–2) to physiologic forms 1 and 2 and susceptible (3–4) to physiologic forms 3, 4 and 5. Goldfoil C. I. 928 is highly resistant (0) to physiologic forms 1, 2, 3, and 4 and susceptible (4) to physiologic form 5. A number of varieties were found to be resistant to all five of the physiologic forms.

Physiologic specialization in Sphacelotheca sorghi. L. E. MELCHERS, C. H. FICKE, and C. O. JOHNSTON.

Three years' data on varietal reaction of sorghums to covered kernel smut prove the occurrence of at least 3 physiologic forms of that organism. Eighty varieties, selections, and hybrids representing the principal groups of sorghum have been used.

Varieties of kafir, sorgo, broomcorn, and kaoliang, as well as miscellaneous sorghums, are attacked by all 3 physiologic forms. Form 1 does not attack milo, hegari, and feterita. Form 2 attacks milo and hegari, but not feterita. Form 3 attacks feterita and certain feterita hybrids, but not milo. Certain Red Amber X feterita hybrids and Spur feterita have not yet been attacked by any of the three forms.

Twenty-three varieties show definite differential reaction and have been arranged in seven groups; viz., one group attacked by all 3 forms; one group attacked by only form 2; one group resistant to all 3 forms; one group susceptible to forms 1 and 3 and resistant to form 2; one group resistant to forms 1 and 2 and susceptible to form 3; one group susceptible to forms 1 and 2 and resistant to form 3; and one group resistant to form 1 and susceptible to forms 2 and 3. These groups have been arranged in a dichotomous key for the identification of physiologic forms of covered kernel smut. (Cooperative investigations by the Kansas Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Stem rust infection of Khapli emmer and Hope wheat in Peru. E. V. ABBOTT.

The results of field experiments with Khapli emmer and Hope wheat during 1928 and 1929 have shown that these varieties are susceptible to *Puccinia graminis tritici* in Peru. When grown in an isolated field Khapli matured with only a trace of stem rust, but when exposed to heavy inoculation by planting near more susceptible common wheats 100 per cent of the plants in one-tenth-acre plots rusted. Although the individual rust pustules were relatively small, they were often so numerous on the culms as to coalesce and cause complete girdling.

Hope rusted more heavily than Khapli, numerous pustules appearing on the culms, necks, leaf bases, glumes and awns. Many culms broke when the kernels were still in the milk stage, and the kernels from heads that matured were badly shrivelled.

Although weather conditions during the growing season were very favorable for rust infection, it is not believed that such high virulence can be attributed to this factor alone. In the laboratory the twelve differential hosts of Stakman and Levine have proved susceptible when artificially inoculated. It is concluded that a new and virulent form of *Puccinia graminis tritici* exists in Peru.

Physiologic specialization in Puccinia coronata avenae. H. C. MURPHY.

Single-urediniospore isolations were made from 45 collections of crown rust, *Puccinia coronata avenae* of which 32 were collected on Avena and 13 on Rhamnus. These cultures were each grown on pure-line selections of 33 varieties and species of oats. Eight of these, acting as differential hosts, disclosed the following 9 physiological forms:

Belar—resistant
- Red Rustproof C. I. 1079—resistant
  - College Algerian C. I. 2052—resistant ..... form III
  - College Algerian C. I. 2052—susceptible ..... form VIII
- Red Rustproof C. I. 1079—susceptible
  - Cowra—resistant, form IX; Cowra susceptible ..... form VII

Belar—susceptible
- Iowa No. 69—resistant; *Avena strigosa*—resistant
  - Anthony C. I. 2143—resistant form IV; Anthony C. I. 2143—susceptible ..... form II
  - *Avena strigosa*—susceptible ..... form I
- Iowa No. 69—susceptible
  - Iowa No. 102—resistant, form VI; Iowa No. 102—susceptible... form V

The 13 aecial collections on 5 different species of Rhamnus represented the following physiologic forms: All 4 collections from *Rhamnus cathartica*, form III; all 6 from *R. lanceolata*, form V; one each from *R. tinctoria*, *R. montana*, and *R. infectoria* were

forms VII, VIII, and IX, respectively. Forms III and V from Rhamnus and I, III, and V from Avena occurred most frequently. Form V was the most widespread and virulent, none of the varieties, pure-line selections, or species being resistant to it. Form I predominated in the Southwestern States. (Cooperative investigations between Iowa Agricultural Experiment Station and Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Dissociations and associations in some strains of Fusarium moniliforme. LEON H. LEONIAN.

Numerous strains of *F. moniliforme* were isolated from corn plants showing symptoms of root rot. Inoculation experiments indicated that these strains possess varying degrees of pathogenicity. After making a single-spore culture from the most virulent of these strains, an attempt was made to dissociate it into as many variants as possible in order more fully to study its scope of fluctuability. As a result of this, some fifty variants were separated from this one strain. After discarding the less-distinct types, there still remained 25 variants easily distinguishable from one another by macroscopic appearance and often by their degree of pathogenicity. The stability of the types was found to be only relative and highly variable, as a given variant would split into new types or revert to the original without any system or regularity. When artificially mixed in petri-dish cultures, usually a perfect blend followed, and colonies resulting from these artificial reassociation duplicated the natural dissociation types observed in plate cultures. When a purple form of these variants was mixed with an orange form, the latter representing a different isolation and undoubtedly a separate strain, often a perfect blend resulted and it required selection for several generations before the types segregated in pure form. In one instance the blending was so perfect as to produce intermediate and new types not observed before. So far these new types have retained their characteristics despite selection continued for generations.

A physiologic form of Ustilago striaeformis parasitizing orchard grass. W. H. DAVIS.

Ustilago striaeformis which causes leaf smut of timothy has been reported as parasitizing both orchard grass and timothy. However, it is not known whether the smut organism which parasitizes these two hosts is one physiologic form, as commonly believed, or a composite species of two physiologic forms.

This investigation was undertaken to solve:

1. Will chlamydospores removed from orchard grass, after-ripened and germinated, infect seedlings of timothy (*Phleum pratense*)?

2. Will chlamydospores removed from timothy, after-ripened and germinated, infect orchard grass?

It is to be noted that reciprocal inoculations with chlamydospores of *U. striaeformis* from the two grass hosts failed to show infections, while inoculations within the host species produced the disease and that the smut organism parasitizing orchard grass is a physiologic form differing from that parasitizing timothy.

Physiologic specialization in Phlyctaena linicola. H. A. RODENHISER.

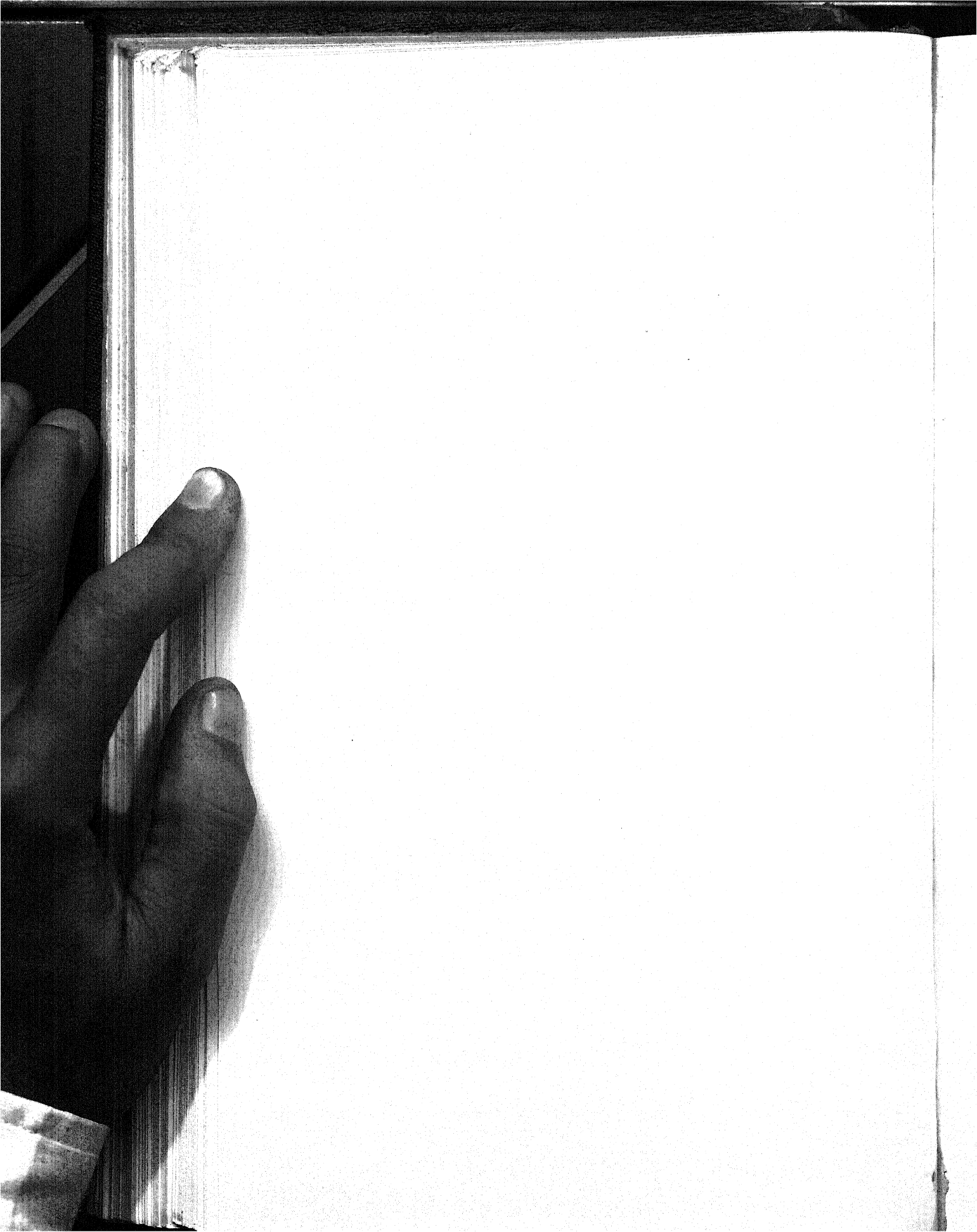
Phlyctaena linicola comprises several physiologic forms. Four forms have been isolated from flax grown in different localities in Minnesota and many more have been isolated from sectors that appeared in colonies on artificial media. Colonies of monosporidial lines can be distinguished by color, topography, surface, consistency, type of margin, the degree to which they produce conidia and by differences in the amount of growth at different temperatures.

Five varieties of flax were inoculated in the greenhouse with 4 culturally distinct monosporidial lines but there were no observable differences in pathogenicity. In the field, however, there were differences in the degree of infection caused by a monosporidial line which produces conidia abundantly, and by another which arose as a sector in a growth therefrom. The range of susceptibility to pasmo in a large number of flax varieties and selections was very wide. (Cooperative investigations between Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Minnesota Agricultural Experiment Station.)

Physiologic specialization in Puccinia triticina in Holland. S. J. WELLENSIEK.

Among collections of the orange leaf rust of wheat from 5 different localities in Holland, physiologic forms 11, 14, and 15 were evidently found to be present. Form 11, therefore, seems to be widespread, both in U. S. A. and in Europe, while the other forms of Mains and Jackson seem to be restricted to U. S. A. and Scheibe's forms to occur widely distributed in Europe.

The wheat varieties usually grown in Holland were all found to be moderately to highly susceptible to all 3 physiologic forms occurring in Holland. Some of the foreign varieties, however, gave a negative reaction with these 3 forms. Breeding for rust resistance, therefore, must be done by crossing foreign varieties with the Dutch ones.



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FUNGICIDAL EFFICIENCY OF CHEMICAL DUSTS CONTAINING FURFURAL DERIVATIVES

C. S. REDDY[1]

Uppal (9), in toxicity studies, showed that the furyl radical was no more toxic than an alkyl radical; and Flor (1), in preliminary studies, found that furfural in dilute solutions offered little promise as an effective fungicide. However, the latter author found that certain organic metallic salts derived from furfural showed high lethal action on fungous spores. Howe and Cation (3) showed that furfural had exceptional properties as a wood penetrant. Recent investigations (2, 6, 7, and 8) also have shown that certain organic compounds of mercury, in comparison with previous standard seed disinfectants, were mild in their action on the host, but efficient in the control of some of its seed-borne diseases.

These investigations led to a study of furfural as a source of organic radicals for metallic compounds, and the applicability of these compounds containing the furan ring as seed disinfectants for the control of corn diseases.

METHODS

Preliminary tests quickly showed that the most promising furfural materials were those containing mercury. Compounds of this type employed by the writer were mercury furoate, mercury furacrylate, mercury dithiofuroate, and others, made by definite procedures, of which the exact compositions were not determined. These toxic reagents were diluted with such fillers as asbestine, calcium carbonate, atomic sulphur, Kieselguhr, and talc.

The compositions of the fungicides studied are:

A1 —4 per cent mercury furacrylate + 96 per cent talc.
A2 —4 per cent mercury furacrylate + 25 per cent sodium furoate + 71 per cent talc.
A4 —8 per cent mercury furacrylate + 92 per cent talc.

[1] Research Fellow, Crop Protection Institute, 1927, on utilization of furfural products as seed disinfectants, stationed at the Plant Pathological Laboratories, under the direction of Dr. I. E. Melhus, Iowa State College, Ames, Iowa.

A6 —8 per cent mercury furacrylate + 25 per cent hydrofuramide + 67 per cent talc.
A¹4 —24 per cent copper furacrylate + 76 per cent talc.
B1 —4 per cent mercury furoate + 96 per cent talc.
B4 —8 per cent mercury furoate + 25 per cent sodium furoate + 67 per cent talc.
B¹2 —10 per cent copper furoate + 20 per cent sodium furoate + 70 per cent talc.
B¹5 —10 per cent copper furoate + 5 per cent arsenous acid + 85 per cent talc.
D5 —5 per cent Paris green + 13 per cent furfural precipitated on 72 per cent talc by ammonia + 10 per cent sodium furoate.
E2C —10 per cent toxic reagent on talc made as follows: 100 cc. furfural were treated with 25 gm. sodium hydroxide and 90 cc. water. When the reaction was completed, 40 cc. ammonium hydroxide were added and the mixture was allowed to stand over night. Seven hundred and twenty gm. talc were suspended in 6 liters of water and the preceding mixture was added. With constant stirring, 80 gms. mercuric chloride dissolved in water were added and the talc and precipitate were allowed to settle. The supernatant liquid then was decanted. What remained was dried and pulverized.
E2C + —Same as E2C except that the mercuric chloride was increased to 100 gm.
E3 —5 per cent. toxic reagent (same as toxic reagent in E2C) + 10 per cent sodium furoate + 85 per cent talc.
G1 —Experimental dust G1 was made by uniting $HgCl_2$, hydrofuramide, and an inert filler such as talc. In the following experiments, it was made as follows: 10 grams hydrofuramide dissolved in acetone were added to 100 gms. talc and mixed. Twelve grams $HgCl_2$ dissolved in acetone were added, mixed, dried, and pulverized to such fineness as to adhere evenly to seed corn.
G3 —same as G1 but has, in addition, 10 per cent sodium furoate.
G7 —Half strength of G1 + 10 per cent sodium furoate.
G8 —One-half strength G3 + 5 per cent hydrofuramide.
67 —10 per cent mercuric chloride + 90 per cent of a mixture of furfural precipitated on Kieselguhr by ammonia.

Hereafter, these fungicides will be referred to by the designation preceding each fungicide, as shown above.

The first consideration in these studies was to obtain a large number of known compounds derived from furfural, especially those containing metals that were not too expensive to be used as seed-treatment materials. To this number were added similar compounds made by the writer. The latter compounds had certain advantages over the former in that they were made for a particular use, could be precipitated directly on the filler in the process of making, and were sometimes previously unknown compounds that were easily made.

In order to determine whether or not the radicals derived from furfural had special merit, they were compared with similar compounds having other organic radicals. The initial experiments were, for the most part, simple biological tests conducted by means of a constant-temperature germinator containing sand trays measuring 20 inches x 20 inches x 3 inches each. One-half inch of sand was spread on the bottom of each tray and covered with a musin cloth. The sand and cloth were made moderately wet and the corn was laid on the cloth in rows with the germ side up. Glass was placed on the top of each tray to retard loss of moisture and assure germination in a saturated atmosphere. Many materials were eliminated, because these simple tests showed that the development of *Diplodia zeae* (Schw.) Lev. on corn seed was not inhibited by them.

In the subsequent preliminary experiments, except for a few semifinal tests in soil in the greenhouse, extensive use was made of the visible-root sand-culture method (5). In these trials "nearly disease-free" seed and seed naturally infected with *Diplodia zeae* were used.

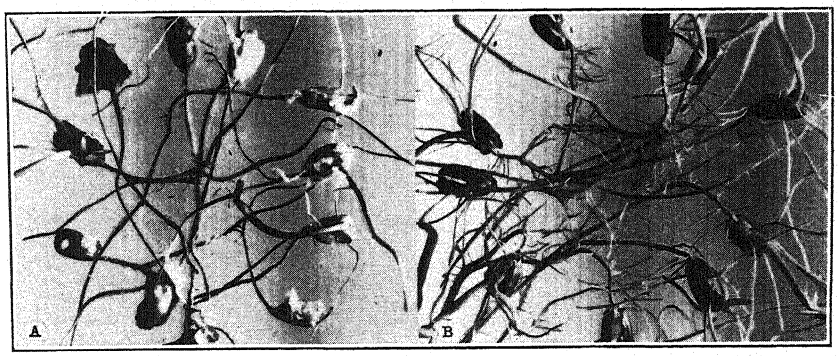


FIG. 1. Seedlings from the sand-tray germinator grown from Diplodia-infected seed corn. A, nontreated; B, treated with G1 dust fungicide.

The purposes of the preliminary experiments were to eliminate a large number of the less suitable disinfectants from expensive field trials and to determine approximately the proper strengths of the disinfectants that were to be tested further. In fact, careful manipulation of the preliminary biological tests seemed to be the most important phase in the development of this new seed disinfectant.

By using the "visible-root sand-culture method," conditions surrounding the germination and growth of the seedlings were largely controlled and it was possible at all times to note the correlation between the presence of disease or injury by the disinfectant and the amount of vegetative growth above the soil. (Fig. 1.) The final test to determine the most ef-

fective seed corn disinfectants was by comprehensive field experiments in which field stands and yields were recorded. Experiments were conducted at 15 separate places in Iowa and two in Illinois, so that the effects of the fungicides were measured under many conditions of corn growth. At Ames, Iowa, a typical experiment with each lot of seed consisted of ten replications of a 30-hill row planted with treated seed paired with 30-hill rows planted with nontreated seed. In each experiment, the number of fungicides was someaimes increased to nine without increasing the number of control rows. Experiments were conducted on several strains of corn, some of which were separated into lots predominantly infected with each of the dry-rot organisms, namely, *Diplodia zeae, Gibberella saubinetii* (Mont.) Sacc., and *Basisporium gallarum* M. Moll. Planting was by means

TABLE 1.—*Effect of a mixture of sodium furoate and furfuryl alcohol in different dilutions on the germination, presence of fungi, and the subsequent growth of the diseased seed corn. Ames, Iowa, 1927*

Ratio of mixture[a] to water	Approximate ratio of furfural to water	Percentage of germination	Percentage of kernels with fungi present	Condition of seedlings
Check	.....	90	70	normal
Check	.....	100	90	normal
Check	.....	90	90	normal
1:0	1:1	0	100	none
1:1	1:3	0	100	none
1:3	1:7	0	70	none
1:7	1:15	80	0	weak
1:15	1:31	100	0	very strong
1:31	1:63	100	0	normal
1:63	1:127	90	50	normal
1:127	1:255	100	50	normal
1:255	1:511	100	50	normal

[a] 100 cc. furfural, 100 cc. water, and 30 gm. sodium hydroxide.

of hand planters, and, to insure uniformity, the kernels were counted for each hill.

The importance of reducing the number of fungicides used in field experiments will be appreciated when it is stated that, even after eliminations, 16 acres were planted at Ames in 1927 and 22 acres in 1928.

EXPERIMENTAL RESULTS

Laboratory experiments. The mixture of compounds obtained from furfural by means of the Cannizzaro reaction (4) was used in different dilutions for treating Diplodia-infected seed. By this reaction, principally,

TABLE 2.—*Effect of experimental dust fungicides on Diplodia-infected seed corn as shown by percentage of germination and by percentage of diseased seedlings. Ames, Iowa, 1927*

Treatment	Percentage of germination after				Percentage of diseased seedlings after		
	3 Days	7 Days	8 Days	11 Days	7 Days	8 Days	11 Days
Check	0	90	90	90	10	30	40
A2a	20	70	70	70	10	10	20
A3	20	70	70	70	0	0	0
A4	60	80	80	80	0	0	0
A5	10	80	80	80	0	10	10
Check	0	80	80	80	30	40	80
A6	20	90	90	90	0	0	0
AC1	0	90	90	90	0	0	0
AB2	20	90	90	90	10	10	20
Check	0	70	70	70	30	30	50
ABC1	10	90	90	90	0	0	10
A1 + A[14]	20	90	90	90	0	0	20
Check	20	70	70	70	20	40	40
B1	40	90	90	90	0	0	10
B2	60	90	90	90	0	10	20
B3	20	70	70	70	0	0	10
B[12] + B[15]	40	80	80	80	0	0	30
Check	20	100	100	100	10	30	40
D5	40	90	90	90	10	20	20
57C	80	90	90	90	0	0	20
67	0	70	70	70	0	0	10
Check	40	70	70	70	10	20	60
E2	50	90	90	90	10	10	10
E2a	30	90	90	90	0	0	0
Check	40	90	90	90	0	20	20
E2b	20	90	90	90	0	0	0
E2c	50	90	90	90	0	10	30
E3	80	100	100	100	10	20	60
G1	10	100	100	100	0	0	10
G3	20	90	90	90	10	10	10
Check	20	90	90	90	10	10	20
G4	20	80	80	80	20	20	60
G5	40	70	70	70	20	30	50
G6	20	80	80	80	20	30	50
Check	20	100	100	100	10	30	50
G7	30	80	80	80	0	0	0
G8	30	100	100	100	10	10	20

sodium furoate and furfuryl alcohol are obtained. In the procedure 30 gm. sodium hydroxide, 100 cc. of water, and 100 cc. of furfural were used. The dilutions were made after the reaction was completed. The seed corn in units of ten kernels was soaked in the dilutions one and one-half hours, dried over night, and placed on the germinator (Table 1).

Table 1 shows that the solutions of the proper dilutions had a fungicidal effect on organisms associated with the germination of Diplodia-infected seed. When the solutions were too concentrated, they killed or injured the seed and allowed fungi to grow, when too dilute, fungi developed on the germinating kernels. It is of interest to note that fungi developed when the treatment was too strong or too weak, but did not develop within a rather wide range of intermediate strengths.

Table 2 gives the results of an experiment to determine the value of some of the experimental dust fungicides, using the sand-tray germinator. Diplodia-infected seed was used in units of ten kernels each.

In table 2 the data on the percentage of diseased seedlings indicate which dust disinfectants, under the conditions of this experiment, affect the development of seedling blight from Diplodia-infected seed. Likewise, the data on percentage of germination indicate which treatments, if any, may injure young corn plants. The dusts which did not retard germination or injure the germinating seed and at the same time control the disease were retained for further study (Fig. 2). On the basis of these data, certain other dusts may be retained and tried at either stronger or weaker strengths.

Table 3 and figure 3 present data on the effect of treating nearly-disease-free and Diplodia-infected seed corn with nine experimental dust fungicides. These data were obtained by the visible-root, sand-culture method in which five crystallization dishes, containing 15 kernels each, were used as a unit. The controls consisted of two control units of untreated seed in each instance. The series in which nearly-disease-free seed was used and one series in which Diplodia-infected seed was used were exposed to medium temperature conditions (16° to 20° C.). A diseased-seed series was placed under high-temperature conditions (22° to 30° C.) and a similar series under low-temperature conditions (10° to 15° C.).

The data in table 3 and figure 3, B, C, and D, show that treated Diplodia-infected seed corn usually emerged faster than nontreated seed of the same kind.

Figure 3, B, C, and D, shows that, excepting No. 3, all the dusts employed in these experiments increased the number of emerging plants. Number 3 was especially injurious at the low-temperature range.

The data in figure 3, E, indicate that none of the dusts was injurious to good seed corn at the medium-temperature range and that No. 2 was

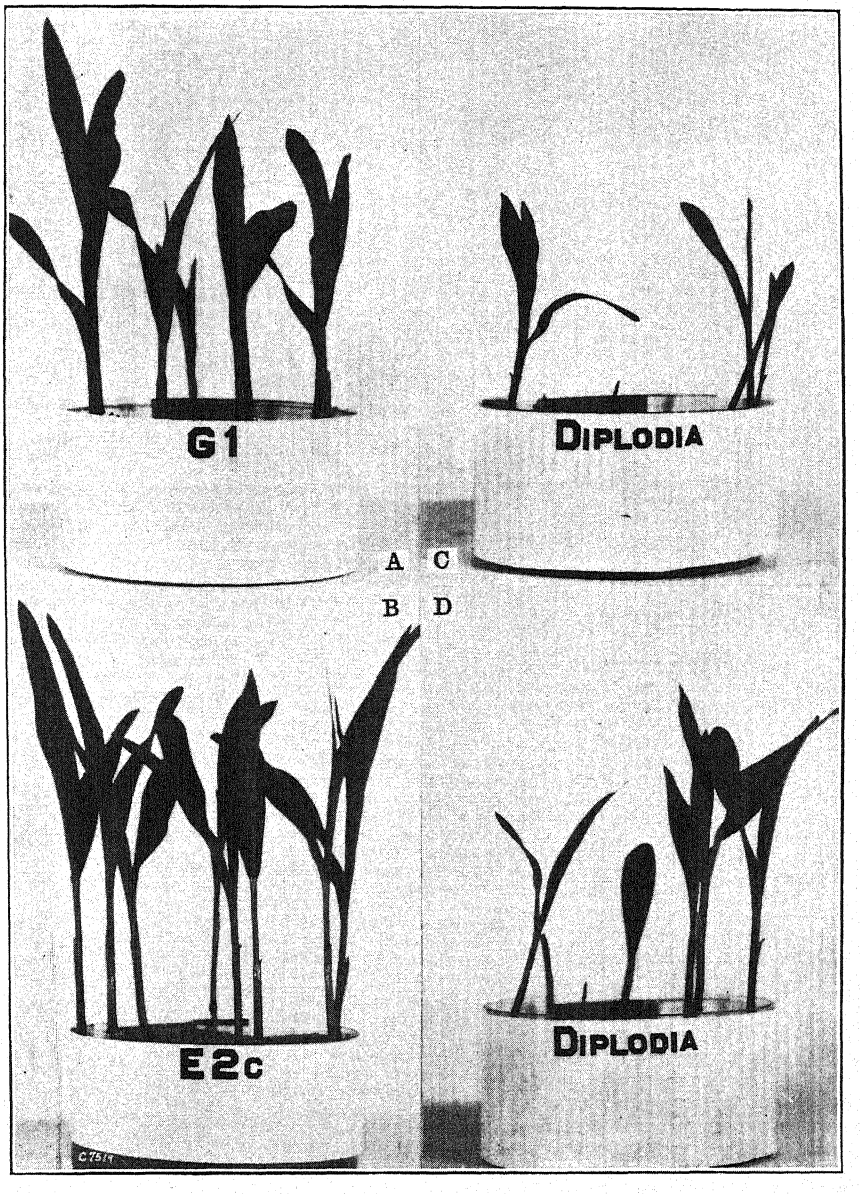


FIG. 2. Seedlings from Diplodia-infected seed corn. A, treated with G1; B, treated with E2C; C, D, nontreated.

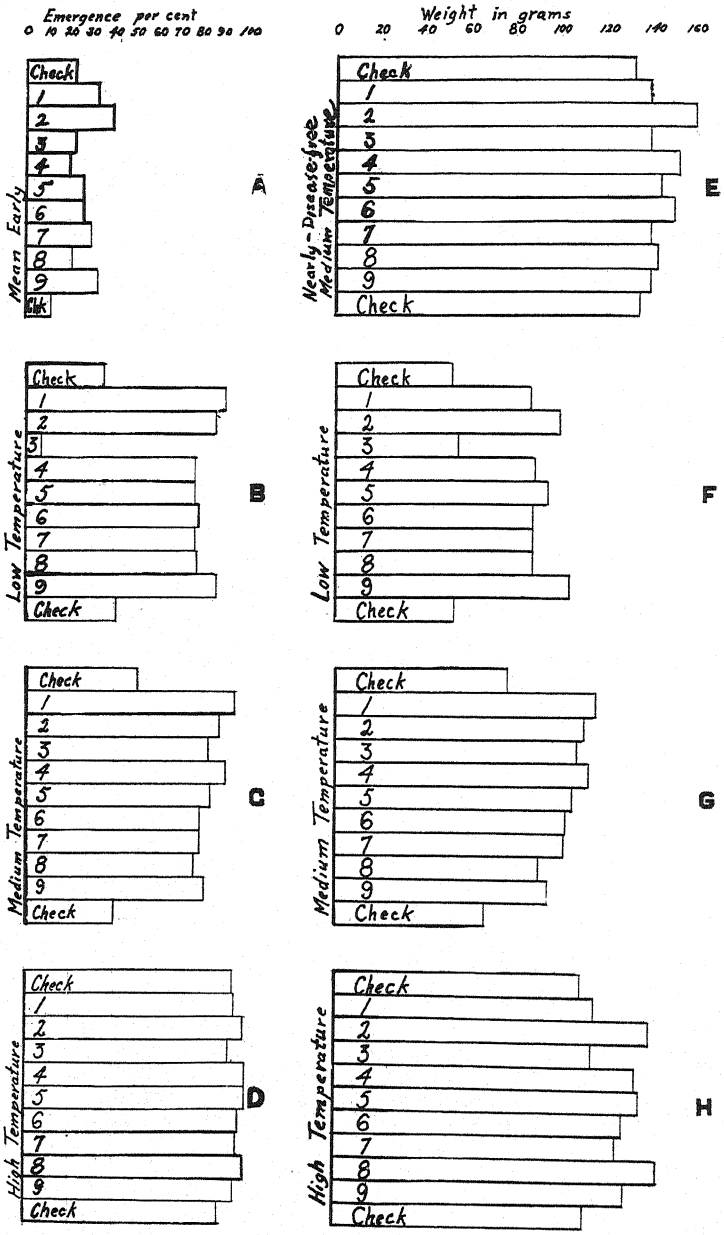


FIG. 3, A. The means of the early-emergence data given in table 3. B, C, D. Graphs showing seedling emergence, under temperature conditions, from Diplodia-infected seed corn, nontreated and treated with nine trial dusts, respectively, table 3. E, F, G, H. Graphs showing the green weight of plants from nearly-disease-free seed corn, nontreated and treated with nine trial dusts and grown under different temperature conditions, table 3.

TABLE 3.—*Data on germination and early growth obtained by the visible-root sand-culture method, to indicate the values of a number of trial dust fungicides for treatment of nearly-disease-free and Diplodia-infected seed corn*

Treatment No.	Nearly-disease-free seed				Diplodia-infected seed									
	Emergence		Green wt. in gms.		Emergence percentage at first and last reading						Green wt. in grams			
					High temperature		Medium temperature		Low temperature					
	No.	Per cent	Total	Corrected to perfect stand	First	Final	First	Final	First	Final	High temp.	Med. temp.	Low temp.	Av. wt.
0	61	81	107	131.6	32	94	32	51	2	36	110	77	52	79.7
1	74	99	137	138.9	37	95	61	95	0	91	116	116	87	106.3
2	69	92	146	158.7	71	99	32	88	16	87	140	111	100	117.0
3	73	97	135	138.7	16	92	52	83	0	7	115	108	55	92.7
4	64	85	129	151.2	16	100	40	91	5	77	134	113	89	112.0
5	72	96	137	142.7	27	100	51	84	3	77	136	106	95	112.3
6	66	88	131	148.9	33	97	39	79	8	79	128	103	88	106.3
7	69	92	128	139.2	25	96	64	79	1	76	126	102	88	105.3
8	71	95	134	141.5	1	99	48	76	15	77	144	91	88	107.7
9	69	92	128	139.1	51	95	31	81	17	87	130	95	104	109.7
0	66	88	118	134.1	12	88	16	40	4	41	112	67	53	77.3

TABLE 4.—*Effect of seed treatments of corn as determined by four replications of 20 hills each of Diplodia-infected seed and two replications of 20 hills each of nearly-disease-free seed. Ames, Iowa, 1927*

Dust disinfectant	Field stand Diplodia-infected		Percentage of increase		Field stand Nearly-disease-free		Percentage of increase or decrease	
	Non-treated	Treated	Stand	Yield	Non-treated	Treated	Stand	Yield
	Number	Number			Number	Number		
A2	71.5	110	53.9	65.3	74	77	4.1	1.4
A6	71.5	104	45.5	45.2	74	74	0	8.8
Ac1	74.5	111	49.0	32.4	75	75	0	− 6.2
ABC1	74.5	98	31.6	36.8	75	77	2.7	9.5
B1	79	111	39.3	45.2	76	74	−2.6	−13.0
D5	79	105	32.9	16.2	76	75	−1.3	− 1.0
67	83.5	124	48.5	43.5	77	71	−7.8	10.6
E3	83.5	105	25.8	33.0	77	72	−6.5	0
E2C	84	109	29.8	30.8	75	74	−1.3	5.0
G3	84	108	28.6	25.9	75	73	−2.7	− 1.6
G8	83	103	24.1	21.2	73.5	71	−3.4	− 6.6
Can. 1:32	83	95	14.5	16.7	73.5	68	−7.5	−11.2
A1+A14	88.5	107	20.9	10.9	73	70	−4.1	3.5
E2C+M2 per cent	88.5	115	30.0	11.5	73	72	−1.4	− 7.2
G1+E2C	75	97	29.3	33.5	68	70	2.9	1.2
Mercurochrome (1 per cent in talc)	59[a]	33	−44.1	−34.2	35[b]	33	−5.7	−12.1

[a] Two replications.

[b] One replication.

outstandingly beneficial. It is of interest to note that, even on nearly-disease-free seed, the injurious character of dust No. 3 was not brought out at this temperature range, a range within its narrow limits of safety. This emphasizes the necessity of trials at different temperatures.

Figure 3, F, G, and H, shows that all the dusts except No. 3 were markedly beneficial to diseased seed. There were indications of benefit from No. 3 only within the medium-temperature range and, therefore, it should be discarded because of its narrow limits of safety. Dust No. 2 was consistently effective at all temperatures and in this experiment was the outstanding dust fungicide. Number 8 was especially satisfactory at high temperatures, while No. 9 was better than No. 8 at low temperatures. Dusts 4, 5, 6, and 7 were uniformly effective, but never the best within any one temperature range.

The results of a preliminary field experiment (Table 4) show that many dust fungicides increase the yield from diseased seed corn, but that not all of them are safe in general practice because of their effect on nearly-disease-free seed.

Tables 5 and 6 show the results of experiments performed by Dr. J. R. Holbert, at Bloomington, Illinois, in which he used one of the furfural-mercury dust fungicides, E2C. The data on the use of a commercial dust are included for purposes of comparison.

The data in tables 5 and 6 show that E2C Dust is a fungicide which increases yields from diseased seed corn, comparable to results from using a commercial dust, and is especially satisfactory from the standpoint of its effect on nearly-disease-free seed.

Table 7 shows the performance of six "furfural" fungicides and one commercial dust fungicide in field experiments at Ames in 1927, on three strains of corn from which a number of nearly-disease-free and diseased lots of seed had been separated.

E2C + gave good results on Diplodia-infected and Basisporium-infected seed, but caused injury to Funk's 176A strain of yellow dent seed corn. This indicates that the modification of E2C, designated E2C +, was not advisable because E2C did not injure the same strain of corn (Table 5) in experiments in Illinois. The fungicide G7, which was approximately half the strength of G1, gave the highest increases in acre yield in three out of five experiments in which it was used with six other disinfectants. This was one of the strong indications that G1 contained more than enough toxic reagent for effective control of the dry-rot seedling blights of corn and that this toxic reagent was effective and noninjurious over a wide range of concentration. Mercury furacrylate, as in A4, gave higher increases in acre yields than the commercial dust in four of the five experiments in which it was used.

TABLE 5.—*Field stands and acre yields of yellow dent corn nontreated and treated with Bayer dust and a furfural-mercury dust (E2C).*[a] *Bloomington, Illinois, 1927*

Kind of seed	Treatment	No. of replications	Field stand		Acre yield		Increase or decrease	Odds
			Non-treated	Treated	Non-treated	Treated	Bu. per acre	
Nearly-disease-free	BD	8	91.9	95.3	46.3	48.4	2.1	7:1
"	E2C	8	92.2	93.2	48.6	49.3	0.7	2:1
"	BD	8	87.0	84.4	56.2	53.5	− 2.7	14:1
"	E2C	8	93.5	93.2	56.5	56.2	− 0.3	9:1
"	BD						− 0.1	1:1
"	E2C						1.7	29:1
"	BD						− 0.1	1:1
"	E2C						− 1.2	2:1
"	BD						3.5	5:1
"	E2C						1.5	13:1
"	BD						− 2.0	1:1
"	E2C						1.5	3:1
"	BD						1.0	1:1
"	E2C						2.5	14:1
"	BD						2.0	3:1
"	E2C						3.7	29:1
Commercial	BD	8	87.5	88.5	50.0	51.1	1.1	2:1
"	E2C	8	86.7	84.4	50.4	50.4		
"	BD	8	80.5	80.2	40.6	44.0	3.4	8:1
"	E2C	8	83.9	84.9	42.9	46.2	3.5	151:1
Diplodia-infected	BD	8	56.5	81.3	32.0	47.9	15.9	506:1
"	E2C	8	56.8	85.9	34.5	51.9	17.4	9999:1
"	BD						11.9	232:1
"	E2C						13.7	35:1
"	BD						12.3	175:1
"	E2C						12.9	61:1
"	BD						15.7	964:1
"	E2C						6.1	21:1

TABLE 5.—(*Continued*)

Kind of seed	Treatment	No. of replications	Field stand		Acre yield		Increase or decrease	Odds
			Non-treated	Treated	Non-treated	Treated	Bu. per acre	
Gibberella-infected	BD	8	71.1	90.1	39.2	53.9	14.7	270:1
"	E2C	8	68.2	87.5	37.3	53.1	15.6	898:1
"	BD						4.6	26:1
"	E2C						2.3	20:1
Fusarium-infected	BD	8	89.0	92.2	49.4	55.0	5.6	132:1
"	E2C	8	87.2	88.0	48.6	52.5	3.9	8:1
Cephalosporium-infected	BD	8	90.6	89.6	51.1	51.1		
"	E2C	8	87.0	84.4	51.3	49.9	− 1.4	4:1
Basisporium-infected	BD	8	76.3	90.6	39.0	44.2	5.2	11:1
"	E2C	8	83.3	92.2	43.3	45.6	2.3	5:1
"	BD						4.3	94:1
"	E2C						2.7	27:1
"	BD						1.4	2:1
"	E2C						6.2	165:1

[a] Toxic reagent is furfural, after undergoing Cannizzaro reaction, and mercuric chloride.

TABLE 6. *Summary of increases and decreases in acre yields following seed treatment with Bayer dust and a furfural-mercury dust (E2C). Bloomington, Illinois, 1927*

Kinds of seed	Fungicide	No. experiments	Av. increase or decrease in bushels per A. for all experiments
Nearly-disease-free seed	Bayer Dust	8	0.46
	E2C		1.26
Commercial seed	Bayer Dust	2	2.25
	E2C		1.75
Diplodia-infected seed	Bayer Dust	4	13.95
	E2C		12.52
Gibberella-infected seed	Bayer Dust	2	9.65
	E2C		8.95
Fusarium-infected seed	Bayer Dust	1	5.6
	E2C		3.9
Cephalosporium-infected seed	Bayer Dust	1	0
	E2C		−1.4
Basisporium-infected seed	Bayer Dust	3	3.97
	E2C		3.73

$B^{12} + B^{15}$ was the only organic copper compound used in the experiments reported in table 7. This compound was ineffective on Gibberella-infected seed. On Diplodia-infected seed it gave about half as much increase as the commercial dust in the same experiments. G1 had ranked high in the preliminary experiments and was used in all the field experiments reported in table 7. The results showed that it was better than the commercial dust in six of the eight field experiments, and the results obtained from the use of G7 showed that G1 might be made more satisfactory by decreasing the percentage of toxic reagent in the dust fungicide.

The effectiveness of two of the furfural-mercury dusts, shown in table 7, is shown more in detail in table 8 and the results are presented graphically in figure 4.

Table 8 and figure 4 show that G1 dust is a promising seed-corn fungicide because it does not injure good seed and materially increases the yields from diseased seed. Major emphasis on perfecting this compound was given in the subsequent year's investigations. Marked improvement has been made, especially in modifying the strength of the toxic reagent. Also, other solvents have been found that can be used in uniting the hydrofuramide and mercury.

Tables 9 and 10 show the effects on field stand and yield resulting from the seed treatment of two different lots of seed corn with a number of dust fungicides.

TABLE 7.—*Field stands and acre yields from yellow dent seed corn nontreated and treated with dust fungicide. Ames, Iowa, 1927*

Kind of corn	Seed treatment	Field stand	Increase in stand following treatment	Mean acre yield	Increase or decrease in acre yield following treatment		Odds
		Per cent	Per cent	Bu.	Bu.	Per cent	
Diplodia-infected U. S. D. A. cross 250 seed	Check	61.5		29.2			
	BD	80.5	30.9	35.3	6.1	20.9	262:1
	E2C+	70.5	14.6	31.7	2.5	8.6	13:1
	G1	79.2	28.7	38.5	9.3	31.8	9999:1
	B4	57.4	−6.8	28.1	−1.1	−3.8	3:1
	G7	77.0	25.2	39.3	10.1	34.6	9999:1
	$B^{12}+B^{15}$	65.0	7.0	32.3	3.1	10.6	23:1
	A4	73.0	18.7	35.5	6.3	21.6	1428:1
Nearly-disease-free U. S. D. A. cross 250 seed	Check	91.3		36.3			
	BD	91.8	0.5	35.6	−0.7	−1.9	3:1
	E2C+	89.3	−2.2	36.0	−0.3	−0.8	1:1
	G1	92.2	0.9	36.4	0.1	0.3	1:1
	B4	82.7	−9.5	35.1	−1.2	−3.3	4:1
	G7	90.7	−0.7	37.6	1.3	3.6	5:1
	$B^{12}+B^{15}$	94.0	2.9	37.1	0.8	2.2	3:1
	A4	90.0	−1.5	37.6	1.3	3.6	5:1
Gibberella-infected Funk's 176A seed	Check	79.8		36.9			
	BD	84.2	5.4	38.2	1.3	3.5	7:1
	E2C+	83.5	4.6	36.8	−0.1	−0.3	1:1
	G1	84.7	7.3	41.1	4.2	11.4	402:1
	B4	80.2	0.4	33.1	−3.8	−10.3	1666:1
	G7	82.8	3.8	39.9	3.0	8.1	16:1
	$B^{12}+B^{15}$	78.3	−1.9	35.6	−1.3	−3.5	6:1
	A4	83.1	4.6	40.9	10.8	4.0	416:1
Nearly-disease-free Funk's 176A seed	Check	94.9		42.2			
	BD	95.3	0.5	40.7	−1.5	−3.6	2:1
	E2C+	93.0	−1.9	36.9	−5.3	−12.6	132:1
	G1	95.5	0.7	41.4	−0.8	−1.9	2:1
	B4	93.7	−1.2	39.7	−2.5	−5.9	6:1
	G7	94.5	−0.4	40.1	−2.1	−5.0	4:1
	$B^{12}+B^{15}$	95.0	0.2	40.4	−1.8	−4.3	3:1
	A4	95.5	0.7	40.2	−2.0	−4.7	9:1
Diplodia-infected Funk's 176A seed	Check	57.0		25.5			
	BD	84.8	48.8	36.5	11.0	43.2	9999:1
	E2C+	84.7	48.6	36.2	10.7	42.0	9999:1
	G1	84.8	48.8	40.7	15.2	59.6	9999:1
	B4	58.2	2.0	25.6	0.1	0.4	1:1
	G7	84.5	48.3	41.6	16.1	63.2	9999:1
	$B^{12}+B^{15}$	70.5	23.7	31.6	6.1	23.9	9999:1
	A4	79.5	39.5	40.5	15.0	58.8	9999:1
Diplodia-infected Murphy strain seed	Check	59.7		29.0			
	BD	75.3	26.3	37.0	8.0	27.6	2399:1
	Sem. Jr.	78.0	30.7	37.3	8.3	28.6	9999:1
	G1	71.0	19.0	34.6	5.6	19.3	276:1
	E2C+	71.5	19.8	36.2	7.2	24.8	1999:1
Basisporium-infected Murphy strain seed	Check	64.7		26.5			
	BD	77.5	19.8	31.7	5.2	19.6	9999:1
	Sem. Jr.	78.6	21.4	30.6	4.1	16.1	1666:1
	G1	75.4	16.5	28.8	2.3	9.0	8:1
	E2C+	78.4	21.2	31.1	4.6	18.0	73:1
Nearly-disease-free Murphy strain seed	Check	91.4		32.1			
	BD	94.9	3.8	33.2	1.1	3.1	7:1
	Sem. Jr.	92.7	1.4	31.9	−0.2	−0.6	1:1
	G1	93.2	2.0	33.3	1.2	3.7	3:1
	E2C+	93.5	2.3	35.0	2.9	9.3	11:1

TABLE 8.—*Yields from nearly-disease-free and diseased seed corn untreated and treated with Bayer Dust and two dust disinfectants made from furfural and mercury. Iowa Agricultural Experiment Station, Ames, Iowa, 1927*

Kind of corn	Dust disinfectant	No. of replications	Acre yields		Increase or decrease		Odds
			Untreated Bu.	Treated Bu.	Bu.	P. ct.	
Disease-free (250)	Bayer Dust	10	36.3	35.6	−0.7	−1.9	3:1
" (Mur)	"	10	32.1	33.2	1.1	3.1	7:1
" (176A)	"	10	42.2	40.7	−1.5	−3.6	2:1
Mean		30	36.9	36.5	−0.4	−0.8	
Disease-free (250)	G1	10	36.3	36.4	0.1	0.3	1:1
" (Mur)	"	10	32.1	33.3	1.2	3.7	3:1
" (176A)	"	10	42.2	41.4	−0.8	1.9	2:1
Mean		30	36.9	37.0	0.1	0.3	
Disease-free (250)	E2C+	10	36.3	36.0	−0.3	−0.8	1:1
" (Mur)	"	10	32.1	35.0	2.9	9.3	11:1
" (176A)	"	10	42.2	36.9	−5.3	−12.6	132:1
Mean		30	36.9	36.0	−0.9	−2.4	
Diplodia-infected (250)	Bayer Dust	10	29.2	35.3	6.1	20.9	262:1
" (Mur)	"	10	29.0	37.0	8.0	27.6	2399:1
" (176A)	"	10	25.5	36.5	11.0	43.2	9999:1
Mean		30	27.9	36.3	8.4	30.1	
Diplodia-infected (250)	G1	10	29.2	38.5	9.3	31.8	9999:1
" (Mur)	"	10	29.0	34.6	5.6	19.3	276:1
" (176A)	"	10	25.5	40.7	15.2	59.6	9999:1
Mean		30	27.9	37.9	10.0	35.9	
Diplodia-infected (250)	E2C+	10	29.2	31.7	2.5	8.6	13:1
" (Mur)	"	10	29.0	36.2	7.2	24.8	1999:1
" (176A)	"	10	25.5	36.2	10.7	42.0	9999:1
Mean		30	27.9	34.7	6.8	24.4	

Tables 9 and 10 show that Bayer Dust used on these two lots of corn ranked first in its effect on yields, although it did not increase the field stand so much as some of the experimental dust fungicides.

Table 11 shows the effect of one of the experimental dust disinfectants on four lots of seed corn, two of which were hybrid seed.

The data in table 11 show that the crossed corn used was not benefited by seed treatment with E2C+, while an open pollinated variety in the same

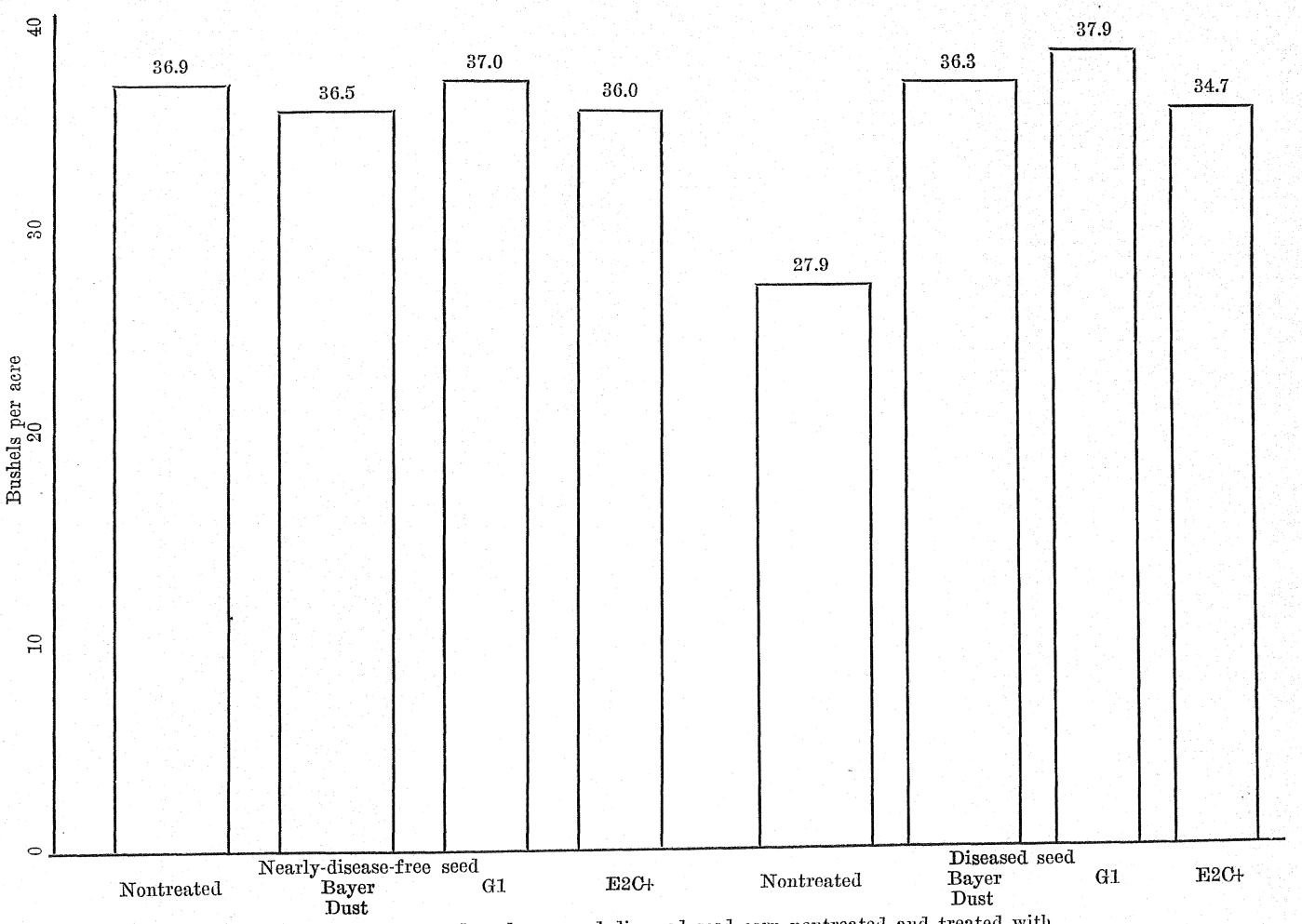


FIG. 4.—Yields from good seed corn and diseased seed corn nontreated and treated with Bayer Dust and two dust disinfectants made from furfural and mercury.

TABLE 9.—*Percentage increases in field stands and acre yields from one lot of poor seed corn following treatment with certain chemical dusts. Ames, Iowa, 1927*

Rank	Dust disinfectants	Percentage increase in		Odds
		Stand	Yield	
1	Bayer Dust	12.3	23.7	9999:1
2	E2C	12.4	15.0	4999:1
3	E2C+	7.5	13.6	207:1
4	G7	4.5	12.7	147:1
5	E2C+G1	13.2	11.9	158:1
6	Sem. Jr.	8.2	11.9	42:1
7	K4	7.5	10.0	33:1
8	Old Homestead	1.7	8.5	11:1
9	E2C5	9.3	7.2	11:1
10	MC1	3.0	5.7	7:1
11	G1	9.6	4.4	9:1
12	$B^{1}2+B^{1}5$	2.6	4.0	3:1
13	A4	2.9	0.3	1:1

TABLE 10.—*Percentage increase or decrease in field stand and acre yield from one lot of poor seed corn treated with certain chemical dusts. Ames, Iowa, 1927*

Rank	Dust disinfectants	Percentage increase or decrease in		Odds
		Field stand	Yield	
1	Bayer Dust	8.0	+15.4	140:1
2	E2C+G1	12.4	+14.2	50:1
3	Sem. Jr.	11.2	+13.1	119:1
4	E2C (half strength)	8.5	+10.9	2249:1
5	MC1 per cent	0.8	+ 8.5	14:1
6	E2C+	12.7	+ 8.2	49:1
7	G1	16.6	+ 3.5	6:1
8	G7	5.1	+ 3.4	10:1
9	E2C	11.8	+ 3.3	4:1
10	$B^{1}2+B^{1}5$	− 2.4	− 0.6	1:1
11	HSD	− 4.4	− 0.9	1:1
12	K4	0.0	− 6.5	9:1
13	A4	0.6	− 4.7	8:1

experimental field was materially benefited by the same seed treatment. Other evidence also indicates that some varieties of corn are benefited more than others by the same seed treatments.

Past experience has shown that seed treatments of Cephalosporium-infected seed (black-bundle disease) have not consistently given beneficial results. As a rule, this disease does not affect germination or early vigor

TABLE 11.—*Effect of E2C + on four lots of seed corn planted at Ames, Iowa, 1927*

Kind of corn	No. replications	Acre yield		Increase or decrease		Odds
		Nontreated	Treated	Bu.	P. ct.	
Nearly-disease-free	10	38.4	42.0	3.6	9.4	37:2
Diplodia-infected	10	34.9	40.3	5.4	15.5	9999:1
Crossed corn A	21	39.3	37.5	−1.8	−4.6	11:1
Crossed corn B	21	38.3	38.7	0.4	1.0	2:1

of the seedlings. Table 12 shows the results of a seed-treatment experiment in which Cephalosporium-infected seed was used.

TABLE 12.—*Effect of dust disinfectant E2C5 on stand and yield of nearly-disease-free and Cephalosporium-infected Funk's Yellow Dent seed corn. Ames, Iowa, 1927*

Kind of seed	No. of replications	Stand		Increase		Acre yield		Increase		Odds
		Treated	Non-treated	No. plants	Per cent	Treated	Non-treated	Bu.	P. ct.	
N. D. F.	10	278	266	12	4.5	19.0	19.3	0.3	1.6	2:1
Cephalosporium-infected	10	224	190	34	17.9	23.4	19.4	4.0	20.6	484:1

In this experiment, the treatment has been of decided benefit to Cephalosporium-infected seed, increasing the field stand 17.9 per cent and the yield four bushels per acre, or 20.6 per cent.

Experiments on sweet-corn-seed treatment were conducted at two places in Iowa in 1927. The experimental method was similar to that used with dent corn at different places in the State. The seed was the same as that used for general planting by the canning company cooperating in the experiment.

The sweet-corn tests at Story City were replicated four times for each treatment. The variety grown in these tests was Golden Bantam. Scarcely any rain fell from the time of planting to harvest time. Moisture was outstandingly the limiting factor for yield and for this reason it was an unfavorable year to determine the value of seed-treatment materials.

The stand and yield data are presented in table 13.

In corn-seed treatment experiments there is usually a high correlation between increases in field stand and in yield. Table 13 shows that, in this experiment, the largest increase in field stands were not followed by the

TABLE 13.—*Data on field stands and acre yields of Golden Bantam sweet corn, prime for canning, from seed-treatment experiments at Story City, Iowa, 1927*

Dust disinfectants	Field stands		Acre yields		Increase or decrease	
	Non-treated No.	Treated No.	Non-treated Lbs.	Treated Lbs.	Stand P. ct.	Yield P. ct.
Sem. Jr.	998	1037	4938	5667	3.9	14.8
Sem. filler	998	980	4938	5208	- 1.8	5.5
G1	998	1152	4700	4842	15.4	3.0
Talc	1049	1106	4700	4958	5.4	5.5
E2C+	965	1032	4700	4942	6.9	5.2
E2C	965	986	4350	4422	2.2	1.7
Bayer Dust	938	957	4350	4210	2.0	-3.2
E2C+G1	911	1026	4350	4250	12.6	-2.3

largest increases in yield. These unusual results are probably due to drought during this season.

The sweet-corn plots at Grimes, Iowa, were not harvested; hence only field-stand data are presented in table 14.

TABLE 14.—*Effect of dust seed treatments on field stands of narrow-grain sweet corn (var. Evergreen) planted at Grimes, Iowa, 1927*

Dust disinfectants	Average of two checks		Increase or decrease
	Nontreated	Treated	
	No.	No.	P. ct.
Sem. Jr.	742	755	1.8
Sem. filler	742	656	-11.6
G1	742	812	9.4
Talc	742	730	- 1.6
E2C+	742	799	7.7
BD	677.5	676	- 0.2
E2C+G1	672	750	11.6

In this experiment the combination of E2C and G1 proved better than either one alone or any of the other disinfectants used.

Experiments were conducted in districts 2, 5, 7, and 10 in connection with the Iowa State yield tests to determine the seed-disinfection value of Bayer Dust, G1, and E2C+. In each of the four districts where these test fields were located, a composite was made with seed obtained from 12 separate farmers to represent average seed not specially selected. From each of the four composite lots four plantings were made, the first of which was

treated with Bayer Dust, the second with G1, and the fourth with E2C+. The third planting was of nontreated seed. In each row was planted the same number of seeds from each of the 12 separate farmers.

Each of four separate lots of seed, entered regularly in the corn yield test by the several growers, was divided into four portions and given the same treatments as the composite lots. These were planted in the same four districts as the composite lots and in similar manner.

The results obtained are shown in table 15 where the acre yields in bushels are given for each trial dust and for the nontreated, or check seed, in each district, together with the average yield for all the districts. No significant differences in yield between treated and nontreated composite lots of seed were obtained. Seed treatments did result in increased yields, however, when used with the four regular entries in the yield test; increases ranging from 0.74 to 2.08 bushels were obtained, each of the treated samples yielding more than the nontreated lots.

TABLE 15.—*Acre yields in bushels obtained from nonselected and specially selected seed nontreated and treated with two furfural and one commercial dust*

Kind of treatment	Acre yields in bushels				
	Dist. 2	Dist. 5	Dist. 7	Dist. 10	Average
Composite seed:					
Bayer Dust	33.59	44.03	50.86	46.74	43.81
G1	31.43	47.81	51.24	44.64	43.78
Nontreated	31.56	47.18	52.71	46.52	44.49
E2C+	33.91	47.04	51.37	44.10	44.11
Selected seed:					
Bayer Dust	37.59	42.42	51.82	52.96	46.20
G1	35.56	42.14	54.23	52.50	46.11
Nontreated	36.70	40.67	47.18	51.94	44.12
E2C+	35.43	40.57	54.10	49.34	44.86

All the lots of seed used in these experiments were studied by germinator tests to determine the amount of disease present. It was found that all lots were unusually free from infection and could be considered comparable to what was designated in previously reported experiments as nearly-disease-free.

The object of experiments with this type of good seed was to determine the noninjurious nature of the disinfectants used. Increases in yield following treatment of this kind of seed are not expected unless plantings are closely followed by adverse growing conditions.

The results of these experiments show that G1 does not injure good seed and may increase the yields of good seed as much as two bushels per acre.

SUMMARY

Organic compounds of mercury having the organic radical derived from furfural have been discovered especially applicable as fungicidal dusts for the control of the dry-rot seedling blights of corn. These are: (1) The compound formed when mercuric chloride and hydrofuramide are brought together in solution; (2) the compounds formed by adding mercuric chloride and mercuric nitrate to furfural after it has undergone the Cannizzaro reaction; and (3) mercury furacrylate.

The field studies on the effect of these fungicidal dusts involved obtaining yields from approximately 1,000 plots of not less than 30 hills, each, near Ames, Iowa; from about 500 plots in Illinois; and from 320 plots in four counties of Iowa.

A number of varieties, strains, and crosses of corn, as well as lots predominantly infected with each of the dry-rot organisms, were used in investigations.

The field stands and acre yields from Diplodia-infected seed were always increased appreciably by the better treatments, sometimes amounting approximately to 50 per cent. Basisporium-infected seed responded by increases in stand and yield of approximately 10 to 20 per cent and Gibberella-infected seed, five to 10 per cent. The effect on nearly-disease-free seed was not significant, but the good seed responded in each instance by increases of about two bushels per acre in the State yield contests in four districts.

The most promising of these furfural-mercury-dust disinfectants were given further study in 1928.

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A RICKETTSIA-LIKE MICROORGANISM IN EUTETTIX TENELLUS (BAKER), THE CARRIER OF CURLY TOP OF SUGAR BEETS

OLIVE SWEZY AND HENRY H. P. SEVERIN[1]

INTRODUCTION

The presence of Rickettsia or Rickettsia-like microorganisms has been recorded from a wide variety of insects within recent years, both as intestinal and as intracellular parasites. Some of these have been connected with various diseases, though the exact relations between them are still obscure in most cases. The majority of them, however, thus far bear no such ill repute. In view of the constantly-increasing importance of insects as carriers of disease, especially in plant pathology, any microorganisms which they harbor may well be made the subject of careful investigation. The presence of such bodies in the beet leaf hopper, *Eutettix tenellus* (Baker), the carrier of the curly-top disease in sugar beets, thus presents a problem which cannot be overlooked in a search for the causal agent of this disease.

Early investigations on the causal agent of curly top were confined mainly to studies of the plant; hence, when the present work was begun, attention was largely given to conditions in the insect which carries this disease. Earlier investigators found no organism associated with curly top in the plant and it has been the consensus of opinion that none exists. Smith and Boncquet (7) isolated and described a bacillus from the lesions produced by curly top but found the same organism in healthy sugar beets and concluded that it had no causal connection with the disease.

Earlier students of the problem were hampered by an inability to produce the disease by inoculation in a sufficiently large number of cases to make experiments practicable. This difficulty was overcome by methods perfected by Carter (2, 3, 4) and Severin and Swezy (6), who thus opened the way for more intensive experimental work than had yet been done. The first result of this improved technique is found in the work of Severin and Swezy on the filterability of the causal agent, proving that both the infective macerated bugs and infected beet juice are capable of producing the disease when passed through fine Berkefeld filters. Coincident with this the present work was begun. Assuming the possibility of a somewhat complicated life cycle for the causal agent which might have a visible stage in the digestive canal or blood of the insect, two lines of attack were fol-

[1] We wish to acknowledge the assistance received from Mr. W. R. Lyon of the University Medical School in carrying on the cultures that were so important to the success of this work.

lowed. The first line consisted of a study of the infective insect, both in fresh smear preparations and in fixed and stained sections of the body and its various organs. The second consisted of cultures made from the crushed insects and beets which had been filtered.

MATERIAL AND METHODS

The methods for conducting filtration experiments on both the insect and the sugar beet were similar to those used by Severin and Swezy (6) in their earlier studies on the filterability of curly top.

For examination of the beet leaf hoppers, various methods of fixing and staining were used, including most of the standard methods of cytological, bacteriological, and protozoological work. Considerable difficulty is offered by the chitinous exoskeleton of these insects, but this was overcome in part by using those adults which had recently moulted. With these, fixation and sectioning offer no difficulties. It was not always expedient, however, to confine the work to these individuals, though they gave the most successful results, and attempts were made to section the insect without removing the chitin. This, of course, resulted in the loss of much material but a sufficient number of good sections were secured to give the required data.

In addition, the intestinal canal and salivary glands also were dissected out, fixed, sectioned, and stained. Smear preparations were made of these organs and of the blood. A fairly satisfactory method of examining the stomach was found in removing the intestinal tract and flattening it between a slide and cover slip or two slides, with considerable pressure, being careful to avoid crushing the organs. If these are allowed to dry slightly before being placed in the fixing fluids the preparation will adhere to the slide and may be stained by the usual methods.

In making cultures of both the leaf hopper and the sugar beet the same methods were used as described for earlier filtration experiments (Severin and Swezy (6)). The leaf hoppers were macerated in a fluid consisting of one-half steam-extracted beet-root juice and one-half five per cent beet sugar. Enough fluid was added to make 100 cc. for filtering. After this material was passed through a fine Berkefeld (W) filter, cultures were made by using 1 loop of the filtrate and 16 cc. of the prepared culture medium and the test tubes sealed with a preparation of equal parts of paraffine and vaseline. Several media were tried and some cultures were made by using steam-extracted root juice alone. The most successful medium was a preparation containing hypophysis. This was made as follows:

Egg white		Locke's solution	.9 per cent
Hypophysis		NaCl	8.5 per cent
Dextrose	.5 gr.	$NaHCO_3$	.05 gr.

Magnesium chloride			.0005 gr.
H_2O	1 liter	KCl	.042 gr.

Subcultures were made at varying intervals, using 1 loop of the original culture to 16 cc. of the sterile culture medium.

When sugar beets were used they were ground in a meat grinder, and the juice was strained out through cheesecloth and filtered. Cultures were made of the filtrate in the same manner, using the same proportions as with the leaf hopper. In all cases the cultures were studied by making smears of the contents, fixing immediately in Regaud's or Schaudinn's fixing fluids, and staining with Giemsa. Smears also were made of the filtrate immediately after filtering and were treated in the same manner. In preparing these cultures both the noninfective and infective insects were used and both healthy and diseased beets.

MICROORGANISMS IN THE LEAF HOPPER

The intestinal canal of the leaf hopper often contains microorganisms in enormous numbers. These are found in greatest abundance in the lumen of the midintestine, though occasionally a few can be found in the oesophagus anterior to the oesophageal valve (Swezy, 8). When only a few are present they are more frequently found in the narrow portion of the midintestine than in the enlarged portion.

They also occur as intracellular parasites. Occasionally a cell in the wall of the stomach will be found greatly distended and its interior completely filled by a mass of bacilli (Fig. 1, A). In other cases a few only of the microorganisms are present in a single cell. These infected cells are most frequently those in the wall of the midintestine though infected cells are found in other parts of the body, as the fat cells in the dorsal and posterior regions and the cells in the posterior portion of the oesophagus near the oesophageal valve (Fig. 1, B). These organisms are also found in the blood. They are rarely found in the Malpighian tubules. The salivary glands have also been free of infection.

As intracellular parasites they seem to be restricted to the cytoplasm. The only exceptions to this that have been noted have been the hypertrophied cells of the midintestine where all available space in both nucleus and cytoplasm seemed to be occupied.

These microorganisms are present in the intestine of both the infective and the noninfective insect, though the former shows a higher percentage of infection than does the latter group. In 333 noninfective insects 41.4 per cent showed the presence of these organisms, while 475 infective insects showed 69.5 per cent infection. These examinations were of the intestinal tract alone. When sections of the entire body are examined the percentage of infection in the infective insect is somewhat higher, reaching about 84 per cent.

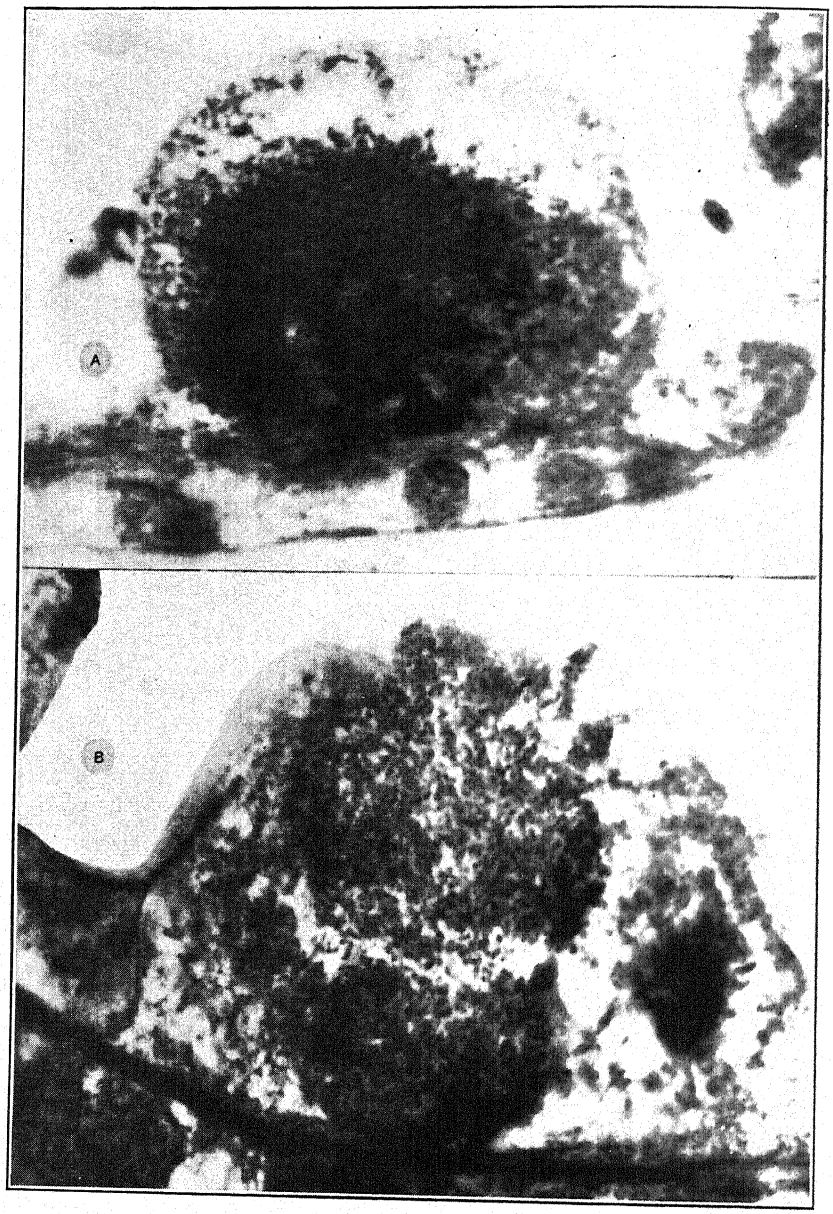


FIG. 1. A. Hypertrophied cell filled with bacilli in the epithelium of the midintestine of *Eutettix tenellus*. B. Hypertrophied cell filled with bacilli in the epithelium of the oesophagus immediately anterior to the oesophageal valve.

In appearance these organisms are diplobacillary in form with ends somewhat rounded, and ranging in size from 0.24 to 3 microns in length. In both staining capacity and sharpness of outline considerable variation is found. In many insects large numbers are present which stain clearly with iron haematoxylin, while others seem to have taken no stain. The same variations may be noted when Giesma is used. An insect is often found with the midintestine filled with a pale grey mass which presents no visible staining reactions, and only the most careful study reveals the fact that these masses are made up of diplobacillary organisms. In this type of organism the outlines, even when stained, are less clear than those of the first type, though they are rather more distinct than in the Rickettsia that have been described from other insects. Both types are found in infested cells but rarely in the same cell.

CULTURES FROM THE LEAF HOPPER

The cultures made from the leaf hopper may be divided into three groups. In the first the entire insect was macerated; in the second the intestinal canal was dissected out and ground up; and in the third the salivary glands alone were used. Large numbers of insects were used in the first group, sixteen hundred to two thousand, making approximately sixteen insects to one cubic centimeter of liquid to be filtered. In the second and third groups dissections were made of from fifty to one hundred insects for each experiment. In the first group ten filtrations were made, beginning on March 19 and ending on July 31. In the second group three filtrations were made and in the third group ten filtrations during the same interval of time. In addition to these, two filtrations were made of macerated leaf hoppers to determine the thermal death point and cultures made from these were examined in the same manner, making a total of twenty-five filtration experiments on the beet leaf hopper.

In most of these experiments smears were made of the filtrate immediately after filtration. These were found to be negative except in one instance where the slides were left exposed to the air for considerable time in drying, and a few sporebearing bacilli were found in the preparation. Cultures were made of the filtered culture medium alone, and slides prepared from these also gave negative results.

Two of the cultures made with salivary glands were examined at the end of twenty-four hours. In the other cases the first examinations were made at the end of two days or later, in some instances. Microorganisms were found in the cultures from each of the twenty-three filtrations as well as the subcultures made from them. Organisms were found in the cultures which had been heated to 65° C. but none in those which had been treated with higher temperatures.

Four attempts were made to secure cultures from the excrement of infective beet leaf hoppers. About three thousand were used each time, five hundred being placed in each of six sterilized test tubes for periods ranging from fifteen to thirty minutes. At the end of this time they were removed and steam-extracted root juice was added to each test tube. These were kept at room temperature for twenty-four hours, then filtered, and cultures made from the filtrate by the usual methods. The first three cultures were examined at the end of one, two, and three weeks and were found negative in every case. In the fourth experiment microorganisms similar to those obtained from the insect were found.

Four filtrations of noninfective beet leaf hoppers were made, using about sixteen hundred insects each time. Cultures were made of the filtrate, using the same methods and examining at the same intervals as in the case of the infective insects. These cultures gave negative results throughout.

The organisms found in the cultures from the infected insects showed a greater pleomorphism than do those in the sections of the insect. Two types predominated, one diplobacillary (Fig. 2; A, B) and the other very short, almost diplococcoid, with intergrading forms between the two. A few cultures showed a giant diplobacillary form, which was regarded as a contaminant but, later, the same organism was found in cultures which had been hermetically sealed in glass tubes immediately after filtering and heated at 70° C. for ten minutes. Their presence under these conditions suggests the possibility that they may be developmental forms of the smaller diplobacillary organism. This is only a suggestion, however.

Both the diplobacillary forms and the diplococcoid forms were found in the same cultures, in some cases in the same tubes and in other cases in tubes which had been made from the same culture but opened on different dates. In many of the cultures the diplococcoid forms appeared before the diplobacillary bodies, while in some cultures the reverse was true.

These organisms are gram-negative and, in Giemsa, show a considerable range of variation in their staining reactions. In some cultures both the diplococcus and the diplobacillary forms take a dark blue stain that is fairly sharp and clear. In other cultures both types show a lesser degree of affinity for the stain and are pale, with their borders not so well defined. They also may be stained with iron haematoxylin.

CULTURES FROM THE SUGAR BEET

The sugar beets used for preparing cultures were those showing an advanced stage of the disease. These consisted of twelve filtrations in which the prepared cultures were kept at room temperatures and two filtrations the cultures from which had been heated to temperatures varying

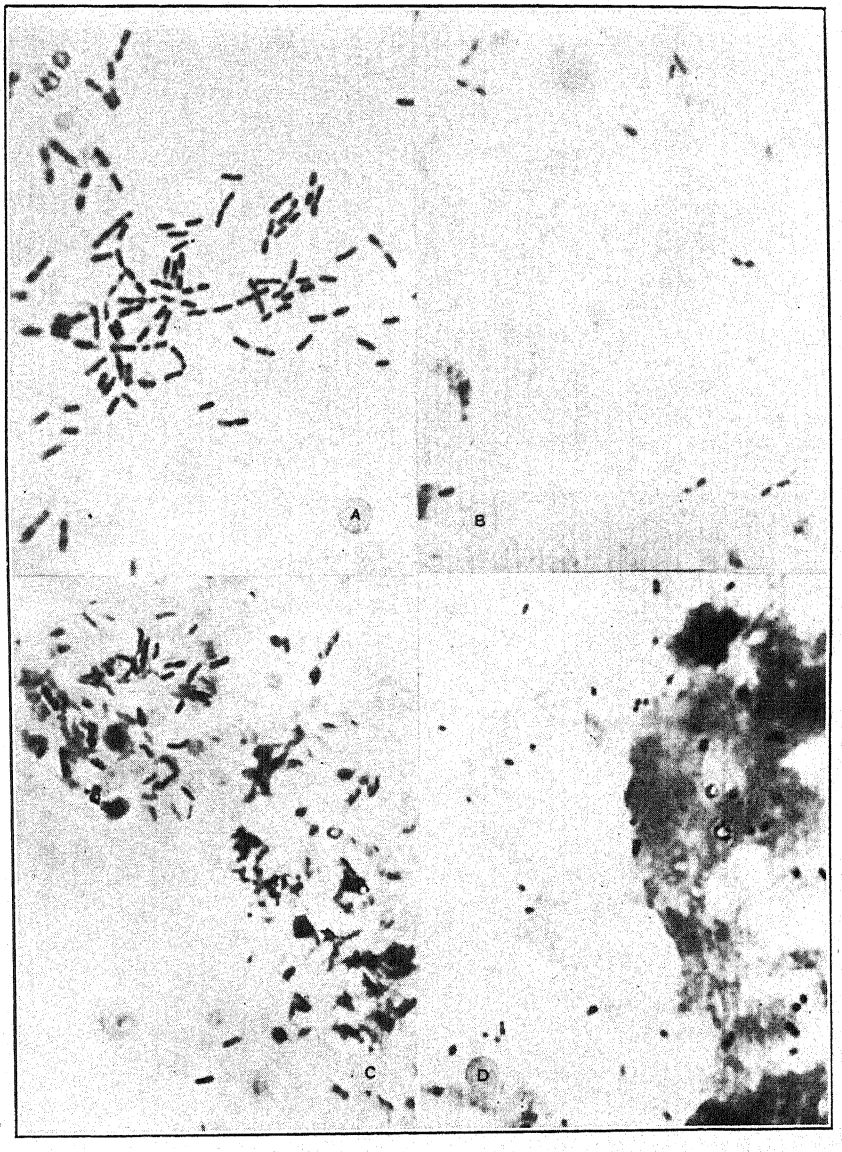


FIG. 2. A. Microorganisms from culture made from filtrate obtained by passing crushed salivary glands of *Eutettix tenellus* through a Berkefeld filter (W). B. Microorganisms from culture made from filtrate obtained by passing crushed intestinal tracts through a Berkefeld filter (W). C. Microorganisms from cultures made from beet-root juice passed through a Berkefeld filter (W). D. Diplococcoid form from culture of filtered beet-root juice.

from 65° to 75° C. to determine the thermal death point, making a total of twelve experiments. One of the first group was examined at the end of one day and the others were examined for the first time at the end of two or more days, the procedure being the same throughout as that followed in dealing with the cultures from the leaf hopper.

In all of the cultures of the first group, and also in those heated to 70° or less, microorganisms were found morphologically similar to those obtained in the cultures from the leaf hopper and showing the same pleomorphism (Fig. 2; C, D). The number of these organisms varied in different cultures, being abundant in some and very scanty in others, though the amount of material used was about the same in each culture tube. The same variation in the appearance of the two types of organisms, diplobacillary and the diplococcoid forms, was found in these cultures with a slightly greater predominance of the latter forms.

DISCUSSION

The group of organisms described under the name of Rickettsia or rickettsia-like bodies has come to include a large number of bacteria-like organisms of still somewhat doubtful classification, which are widely distributed in insects and arachnids. As defined by Arkwright, Atkin, and Bacot (1921), the Rickettsia are characterized by their minute size, usually being 0.2 to 0.5 micron in diameter; their pleomorphism from coccus-like and diplococci to bacillary forms; their resistance to ordinary aniline stains; their loss of gram stain and affinity for Giemsa; their absence of motility; their resistance to cultivation on ordinary media; and their occurrence in the intestine of blood-sucking insects. Hertig and Wolbach (5) further suggested that the term be limited to the organisms of proved pathogenicity. As pointed out by Ward (9), the latter characteristic, as well as size, is of doubtful value for classification.

The position of the organisms found in *Eutettix tenellus* and in the cultures made from it and the infected beet is open to some question, and, for the present, no attempt will be made definitely to allocate these organisms. Their affinities seem to be with the bacteria, however. They possess some of the characteristics of Rickettsia but differ from them mainly in their greater affinity for stains and in their motility. Regarding their pathogenicity nothing can yet be said either for or against their connection with the disease of sugar beets. That the same type of organism may be secured by filtration-culture experiments with the infected beet and infective insect is suggestive of a causal relation between them, however. The rather wide range of variation in size of the organisms present in the cultures is extended when the form small enough to pass the filters is included. That this latter type is an ultramicroscopic one is indicated by two facts: (1)

No organisms have been found immediately after filtering; and (2) none have been found in the numerous sections of salivary glands, though filtered cultures of the glands will give the same forms present in cultures from the entire insect. In making the cultures from the salivary glands the insects are placed in a small vial partly filled with sterile physiological salt solution. The vial is shaken before removing the insects to partially immobilize them, after which they are placed in fresh solution in a flat watch glass under the microscope for dissection. The glands are removed while the insect is immersed in the fluid and removed to a small stender dish containing sterile physiological salt solution. When this is done with care the intestinal tract and other organs of the body are left intact, only the head being removed. With the amount of salt solution used the possibility of contamination is reduced to a minimum, though there is always the chance that a few organisms from the blood may be carried over when the salivary glands are removed from the watch glass. Since the diplobacillary forms are usually present only in the intestinal tract and when found elsewhere are very scanty in numbers, the possibility of the cultures becoming infected in this manner is remote.

The form present in the sugar beet is also an ultramicroscopic one, since no visible organisms have been found in the diseased plant tissues, with one exception; sometimes the cells in the periderm of the beet root show from few to many bacilli similar to those in the cultures.

SUMMARY

It is evident that *Eutettix tenellus* harbors two different organisms, though these cannot be separated on morphological grounds. The differences between these two bacilli lie in the fact that one may be filtered and the other cannot pass through the fine Berkefeld filters; the one being found in infective insects and the other being present in both. This difference is evidently one that will be found closely correlated with differences in the life histories when these shall have been worked out more definitely.

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FURTHER NOTES ON *BACTERIUM TUMEFACIENS* AND ITS HOST RELATIONSHIP[1]

J. Ben Hill, Wm. H. Brittingham, Frances P. Gibbons, and Grace W. Watts

It has been shown by one of the authors (2) that *Bacterium tumefaciens* Smith and Town., in common with several other species of bacteria, migrates through the host tissues in the form of zoögloeae. In the present report the writers wish to assemble several additional facts bearing on the migration of this organism.

To obtain the tobacco stems used in these investigations old plants of the flowering tobacco, *Nicotiana affinis* Moore, were cut back to induce new succulent growth and these new, vigorously-growing shoots, 0.3 to 0.5 cm. in diameter, were inoculated in the internodes 0.5 to 1 cm. from the apex. The pieces of stem containing the inoculum were fixed for sectioning in alcohol-acetic acid-formalin solution, at intervals of 5, 10, 15, 20, 30, and 45 minutes, and 1 hour. This material was imbedded in paraffin and, after cutting, the sections were stained with Flemming's triple stain. Of the strains of *Bacterium tumefaciens* used in these studies "peach," "willow," "euonymus," and "pecan" were originally obtained from the laboratory of Dr. Erwin F. Smith, Bureau of Plant Industry, Washington, D. C., and "Wisc. 2004," "Wisc. 2141," and "Wisc. 2018" from Professor Wm. F. Banfield, University of Wisconsin, Madison, Wisconsin.

MIGRATION OF BACTERIUM TUMEFACIENS IN THE XYLEM VESSELS OF THE HOST[2]

Robinson and Walkden (4) and Hill (2) show that *Bacterium tumefaciens* migrates through the xylem vessels of the host. Hill indicates that the bacteria migrate vertically both upward and downward on gaining entrance to the lumen of the vessel after the wall is broken. It is the purpose of this note to report investigations conducted to ascertain the manner, direction, and rate of migration of *Bact. tumefaciens* in the xylem vessels of tobacco.

Since *Bacterium tumefaciens* is dependent upon the rupture of the walls of a cell to gain entrance to the lumen, a special inoculation technique was

[1] Contribution from the Department of Botany, The Pennsylvania State College, No. 70. Published with the approval of the Director of the Agricultural Experiment Station as Technical Paper No. 478.

[2] Investigation by Wm. H. Brittingham.

developed for the study of migration through the vessels. A scalpel was ground until it was narrow, flat, two-edged, and tapering to a point. By inserting this instrument into the shoot at right angles to the stem axis, it was possible to cut several vascular bundles and bring bacteria into contact with many severed xylem vessels. An effort was made to introduce a maximum dosage of bacteria in each inoculation. By regularly cutting pieces of the stem so that the puncture was nearer one end than the other, the part above the point of inoculation could be distinguished from the portion below.

In every section studied numerous zoögloeae were found in the xylem vessels both above and below the puncture. The tips of these zoögloeae revealed in longitudinal sections of the stem were both rounded and irregular in outline. In those of irregular outline there were frequently several lobes or advancing points. This type of zoögloeal tip is characteristic of zoögloeae occupying relatively large spaces, as has been shown by Haber (1) and Hill (3).

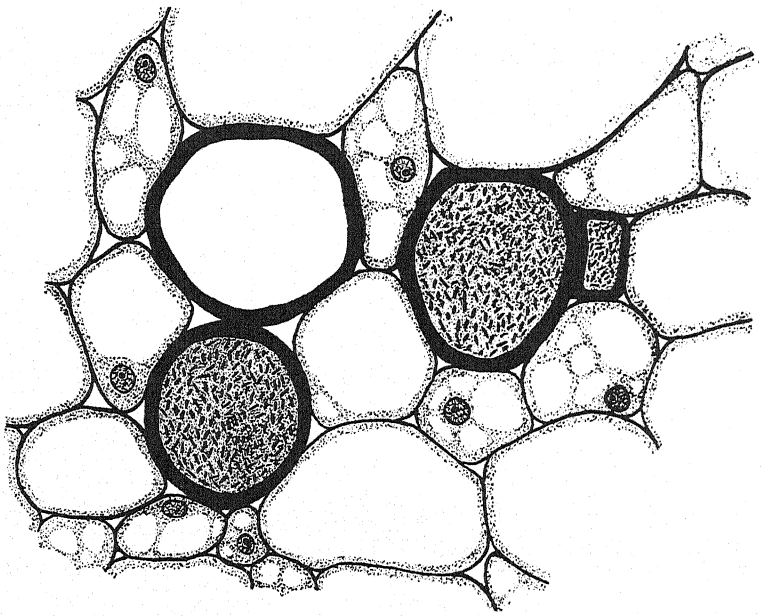


FIG. 1. Transverse section of stem of tobacco showing vessels whose lumina are occupied by zöogloeae of *Bacterium tumefaciens*.

The rates of migration in the vessels were determined from the study of the transverse sections (Fig. 1). Since all of the sections were each 12 microns in thickness, it was easy to calculate the maximum distance from

the puncture which the zoögloeae advanced in the period preceding fixation. It was found that within 15 minutes the zoögloeae advanced 2.378 mm. through the xylem vessels above the puncture and 2.161 mm. through the vessels below the puncture.

COMPARISON OF BEHAVIOR OF BACTERIUM TUMEFACIENS IN TISSUES OF VARIOUS PLANT HOSTS[3]

In connection with a study (2) of *Bacterium tumefaciens* in the tissues of tomato, *Lycopersicum esculentum* Mill., various other plant hosts, such as tobacco and castor bean, *Ricinus communis* L., were used as checks in the investigation. These have provided material for interesting comparisons of the behavior of the parasitic organism in the various hosts.

The zoögloeae of the parasite correspond in size and shape to the intercellular spaces they occupy. Thus in the intercellular spaces of the pith of the tobacco, which are much larger than those of the tomato, the zoögloeae are much larger than those in the corresponding position in the tomato; the advancing ends of the zoögloeae often are irregular, with several lobes or advancing points, while those in the narrow intercellular spaces of the tomato have rounded, blunt ends. Such irregular zoögloeal tips resemble those described above as occurring in the vessels of the tobacco.

The anatomical structure of the host plant probably determines the path of migration of *Bacterium tumefaciens* through the tissues. This appears to be particularly in evidence where there is considerable diversity in the structure of the various hosts. In the stem of the tomato plant there is a subepidermal layer of loose parenchyma tissue two or more cells in thickness, while the tobacco and castor bean have no such layers. In a former paper (2) it was shown that, following inoculation, the subepidermal layer in the tomato plant afforded a path of migration for the parasite. No zoögloeae were observed in the cortex proper of the tomato, although the pith tissue was regularly invaded by the zoögloeae, advancing into its intercellular spaces. In contrast with the situation in the tomato, the tobacco stem appeared to be invaded only by way of the intercellular tissues of the pith. A study of the migration of *Bact. tumefaciens* in the tissue of the castor bean, *Ricinus communis,* shows that the intercelluar spaces of the pith are regularly penetrated by the zoögloeae and that, in addition, zoögloeae penetrate the cortical tissue by way of the intercellular spaces but apparently not the subepidermal layer. The larger size of the intercellular spaces of the cortex of the castor bean, as compared with those of the tomato, is probably related to the use of this tissue as a path of migration in the former and not in the latter host. Following inoculation, the

[3] Investigation by J. Ben Hill.

galls produced on these hosts in a given period are largest on the tomato and smaller on the castor bean and tobacco. The probability of a correlation between the location of the path of migration and the size of galls formed by *Bact. tumefaciens* in its various hosts is suggested by the observed variation in the relative sizes of galls produced on the different host plants as well as by the facts here presented in connection with the anatomical structure and the path of migration.

COMPARISON OF THE MOTILITY OF BACTERIUM TUMEFACIENS IN CULTURE AND IN THE HOST TISSUE[4]

Another of the aspects of *Bacterium tumefaciens* which has been studied is its motility. The question arose as to whether the organism was, as E. F. Smith (5) says, "a small, white, motile, polar flagellate, rod-shaped schizomycete" and whether this could be said of the organism both in artificial culture media and in the zoögloeae in the tissues.[5] The technique of flagella staining was so uncertain that the hanging drop was used as an index of motility. Young tomato stems and beef broth were inoculated with a culture of the "peach" strain of *Bact. tumefaciens.*

At the end of 18 hours bacteria in the hanging drop from the broth showed motility, but the plant juice, squeezed from the surface of the stem cut at the point of inoculation, showed many bacteria, *all nonmotile.* These inoculations in broth and tissue were made simultaneously, and the drops observed in close succession. Over a period of 10 days, twenty-five to thirty observations all confirmed these findings. To make certain that the bacteria-like, nonmotile bodies seen in the plant juice in the hanging drop were bacteria, a plate dilution of a drop of the juice was made, and isolations into beef broth became motile at the end of 18 hours. These same bacteria had been nonmotile in the plant tissue 18 hours before. Also to show the presence of bacteria in the hanging drop of plant juice which showed no motile bodies, twelve tomato plants were inoculated directly with the expressed tomato juice, and galls were formed in every case.

COMPARATIVE STUDY OF THE BEHAVIOR OF CERTAIN STRAINS OF BACTERIUM TUMEFACIENS IN PLANT TISSUE[6]

Investigations of various workers, particularly Smith (5), have shown the existence of a large number of strains of *Bacterium tumefaciens* which

[4] Investigation by Frances P. Gibbons.

[5] This study was the result of a suggestion by Dr. H. R. Rosen, of the University of Arkansas, who, in conversation with the senior writer, expressed the thought that certain parasitic bacteria may be nonmotile in plant tissue.

[6] From a thesis submitted by Grace Weston Watts in partial fulfillment of the requirements for the degree of Master of Science at The Pennsylvania State College, published with the approval of the Dean of the Graduate School.

show a considerable range in virulence. Recent work by Hill (2) in calculating the rate of growth of the zoögloeae of *Bact. tumefaciens* has furnished a new method for a comparison of the strains of this organism.

This investigation was undertaken to secure a comparison of certain strains of *Bacterium tumefaciens* and to determine whether any correlation exists between the virulence of these strains as expressed by gall formation and development and the rate of migration of the bacteria through the tissues. With preliminary work on the virulence of strains as expressed by the rapidity of gall formation as a basis, certain strains, showing a wide range of virulence, were selected for the investigation.

For the preliminary studies, seedling tomato plants about 20 cm. in height were inoculated in the internodes between the cotyledons and the first leaves. Two inoculation punctures intersecting at right angles were made in each plant. Several plants were inoculated with each of the strains and observed daily for several weeks. After some information had been gained concerning the relative virulence of the strains as expressed by the rapidity of gall formation, three strains, peach, willow, and Wisc. 2004 were selected for intensive study. These strains represented the extremes of the range of virulence. Of these, the peach strain gave macroscopic evidence of its virulence by gall formation in from three days, at the earliest, to five days, at the latest. The galls produced by Wisc. 2004 and the willow strains were slower in their development and never attained

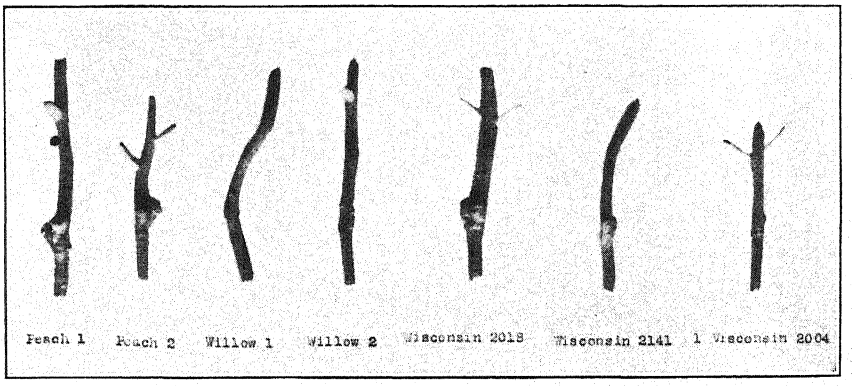


FIG. 2. Stems of tomato showing galls produced by various strains of *Bacterium tumefaciens*. Inoculation June 30, 1927. Photograph July 25, 1927.

the size of those produced by the peach strain. These two latter strains were hardly distinguishable on the basis of the rate of their gall production, both the Wisc. 2004 and the willow producing recognizable galls only after a period of five to twelve days. The largest galls were produced

on plants inoculated with the peach strain and the smallest on those inoculated with the Wisc. 2004 (Figs. 2 and 3).[7]

Since the tobacco plant provides better material for sectioning than does the tomato, the former was selected as a host in the study of the rates of migration of the strains selected for intensive study. For this phase of

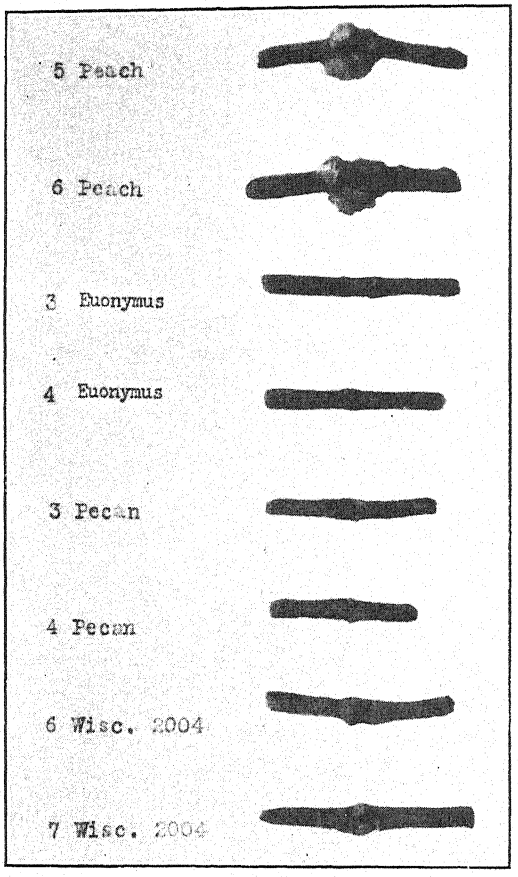


FIG. 3. Stems of tomato showing galls produced by various strains of *Bacterium tumefaciens*. Inoculation July 26, 1927. Photograph August 25, 1927.

the study, inoculation with the peach, willow, and Wisc. 2004 strains was accomplished by making a single stab with the needle. Microscopic study of these sections of the tobacco stems showed the zoögloeae located in the intercellular spaces of the pith. They were growing in most instances in

[7] Acknowledgments are due Mr. C. W. Smith, College Photographer, for assistance in photography.

a vertical direction from the puncture. Zoögloeae were more abundant in the peach and willow than in the Wisc. 2004 strain, although there seemed to be no difference in the size of the individual zoögloea of the different strains.

When zoögloeae were located, measurements were made from the point of puncture to the remotest part of the zoögloeae. The rates of migration calculated (Table 1) are the result of the study not of one or two stems

TABLE 1.—*The comparative rates of movement of different strains of Bacterium tumefaciens in tobacco stems*

B. tumefaciens	Average distance penetrated in 15 min.	Average distance penetrated in 30 min.	Average distance penetrated in 45 min.
"Peach" strain	.7668 mm.	2.13325 mm.	2.2288 mm.
"Wisc. 2004" strain	.5068 mm.	.987 mm.	1.1593 mm.
"Willow" strain	.5355 mm.	.8194 mm.	.79625 mm.

but of many sections from many stems representing several separate inoculations. For example, the rate given in table 1 for the peach strain is an average of forty-nine separate measurements from ten or more inoculations. When zoögloeae were found in identical positions on three successive sections, they were assumed to be the same zoögloea, and only one measurement was made, namely, that of the part that ended farthest from the puncture. Zoögloeae in the broken tissue at the edge of the puncture were not measured unless their remotest ends extended beyond the tissues disorganized by the puncture. Zoögloeae of peach, willow, and Wisc. 2004 were found at distances from the point of puncture, varying from a fraction of a millimeter to several millimeters.

In comparing the results of the studies on migration and those on the virulence of different strains as evidenced by the rapidity and size of gall formation, a direct correlation appears between the virulence of each strain and its rate of migration. In peach, for instance, a large gall is formed during a period of twenty to thirty days, and in Wisc. 2004 the gall formed during the same period is almost negligible (Figs. 2 and 3). An inspection of the results of the studies on migration as indicated by table 1 shows differential rates of migration for the three strains under consideration. These rates of migration are, in general, in direct correlation with the virulence of the strain as expressed by gall formation. The peach strain, which produces larger galls and forms them more rapidly than the

other strains, has a more rapid rate of migration than Wisc. 2004 and willow. This was true every time measurements were taken.

Work done by Hill (2) shows that there is a decided decrease in the rapidity of the rate of migration of the bacteria as they continue their development in the host tissue. A similar decrease in the rate of migration is also found in this investigation. There is an interesting comparison between the rate of migration of the peach strain of *Bacterium tumefaciens* in tomato tissue as worked out by Hill (2), namely, 0.04 mm. per minute, and that of the same organism in tobacco tissue in this investigation, that is, 0.05 mm. per minute, in both instances computed from sections fixed 15 minutes after inoculation.

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THE ZOÖGLOEAE OF *BACTERIUM TABACUM* AND THEIR RELATION TO THE PROBLEM OF THE MIGRATION OF BACTERIAL PHYTOPATHOGENES THROUGH THE HOST TISSUES[1]

J. Ben Hill[2]

INTRODUCTION

Within recent years several contributions have been made relative to the topic of the migration of bacterial phytopathogenes through the tissues of the host. Nixon (12, 13) and Haber (8) showed that *Bacillus amylovorus* Burr. migrates in the form of zoögloeae through the tissues of the stem and leaf of the apple. Beach (4) described the zoögloeae of *Bacterium vignae* Gardn. and Kendr., parasitic in the tissues of the leaves and pods of the lima bean. Hill (9, 10) reported the presence of zoögloeae in *Bact. tumefaciens* Smith and Town. and the rate of migration in the tissues of the stem of the tomato. All of these authors have emphasized the importance of zoögloeae in relation to the migration of these organisms.

Arthur (1), in 1886, in a study of the biology of *Bacillus amylovorus,* considered the zoögloeae observed in liquid culture to be the most characteristic feature in the life history of that organism. Bachman (3), investigating the migration of *B. amylovorus,* said "the bacteria are surrounded by a film of liquid which it seems likely has been drawn from the cell sap."

Robinson and Walkden (19) and also Riker (16, 17, 18) considered the migration of *Bacterium tumefaciens.* The former authors were primarily interested in other phases of the behavior of the organisms and the latter apparently had a misconception of the actual method of migration of the parasite.

When the earlier investigators, Atkinson (2), Beyerinck (5), Dawson (6), Frank (7), Kny (11), Peirce (14), Prazmowski (15), Tschirch (21), and Ward (22) focused their attention on the nodule-forming bacteria of the legumes, among other questions they considered was that of the migration of the causal organism. During the period between 1879, when

[1] Contribution from the Department of Botany, The Pennsylvania State College, No. 69. Published with the approval of the Director of the Agricultural Experiment Station as Technical Paper No. 477.

[2] I am indebted to Mrs. Helen D. Hill and to my colleagues in the Department of Botany at The Pennsylvania State College for criticism of this manuscript. Credit is also due the College Photographer, Mr. Chas. W. Smith, for assistance in photography.

Frank (7) discussed the nodule-forming organism of the Leguminosae, *Bacterium leguminosarum* Frank (*Rhizobium radicicola* Hiltner and Storner) (*Schizia leguminosarum* Frank) (*Bacillus radicicola* Beyerinck) (*Pseudomonas radicicola* Moore), and 1902, when Peirce (14) described the migration of that organism in the bur clover, several investigators described the manner in which these bacteria pass through the root and nodule tissues of various legumes. Notwithstanding the confusion surrounding the whole question of nodules on the roots of legumes and the various opinions as to the nature and even the identity of the causal organism and the diversity of terms used in describing its advance through the tissues, there was considerable agreement among these early authors as to the actual path and method of migration of the causal organism. All agreed that it occupied a position within the lumina of the cells. The advance through the tissues was accomplished by some kind of filamentous mass or structure to which were applied different names, such as bacterial filaments, bacterial tubes, plasmodia, infection threads, infection hyphae, infection tubes, and zoögloeae. Smith (20) gave a fairly comprehensive bibliography on nodule-forming bacteria of the legumes but with very brief abstracts of the papers and with little attempt to correlate their ideas on migration. In the preparation of this manuscript all of the articles cited have been consulted. Though these early investgators entertained a variety of ideas as to the nature and identity of the causal organism of the nodules, many of them both figured and described their conception of the method of migration of the organism. In several instances the organism is described as surrounded by a gelatinous matrix in the form of branching filaments which penetrate the cell walls and pass from cell to cell.

In the first studies on wild fire of tobacco, Wolf and Foster (23) reported that in the early stages of the disease the causal organism *Bacterium tabacum* Wolf and Foster occupies the intercellular spaces of the leaf of the host. In presentation of this report of the study of the zoögloeae and migration of the causal organism of the wild fire of tobacco, *Bact. tabacum*, in the tissues of its host, the objects are: To submit evidence which shows that another parasitic bacterium, though manifesting its presence in the plant tissues by symptoms quite different from some of the forms recently studied, migrates in a manner entirely comparable with those other forms; to make comparisons of the zoögloeae of this organism with those known in other bacterial species; and to show the relation of these zoögloeal structures to the conception of bacterial migration by zoögloeae.

INVESTIGATION

Technique. The culture of *Bacterium tabacum* used in this investigation was isolated from naturally-infected tobacco seedlings grown in Lan-

caster County, Pennsylvania, and was thoroughly tested and known to produce the disease. The microscopic study was based upon material both from the original natural infections and from infections through artificial inoculations on leaves of *Nicotiana tabacum* L. Inoculation of young vigorous leaves of cultivated tobacco, growing in the open during the summer months, was made from profuse growths of *Bacterium tabacum* on agar slants. A quantity of the inoculum was smeared on the leaf and a very fine inoculating needle was pushed through this mass into and completely through the leaf. Material was fixed either in Flemming's weak solution or in alcohol-acetic acid-formalin solution at intervals up to three days from inoculation. The material imbedded in paraffin was cut about 12 microns in thickness both transversely and in a plane parallel with the epidermal surfaces of the leaf. Flemming's triple stain gave good differentiation of both the bacteria and the matrix of the zoögloeae.

The two main points in the technique for the study of the migration of any bacterial form in plant tissue are to secure the material at the proper stage in the migration and to modify the stains to secure the best differentiation of the bacteria and matrix in the tissues. With material grown in the open at summer temperatures, the period found best for showing evidence of migration of the bacteria in the wild fire of tobacco was from 48 to 72 hours after inoculation.

Observations. In the sections prepared from material fixed 24 to 36 hours after inoculation, the bacteria can be seen growing in the mass of tissue lacerated by the needle. In the material fixed 48 to 72 hours after inoculation, the bacteria may be seen in the uninjured tissue surrounding the needle puncture. Zoögloeae have been formed and these penetrate the healthy tissues, passing through the intercellular spaces and in the early stages apparently doing little, if any, harm to the cells of the host (Fig. 1, A, B). After cells have been surrounded by bacteria for an undetermined period, plasmolysis and disorganization of the protoplast are evident (Fig. 2, E). The tissue most frequently penetrated by the zoögloeae is that composed of the parenchyma cells near the smaller vascular bundles of the leaf. Though bacteria were seen in some of the smaller vessels, the invariable injury of the latter leads to the belief that the bacteria gain entrance to vascular elements only when these have been ruptured.

Microscopic study of the sections of tobacco leaves, fixed about 72 hours following their inoculation with *Bacterium tabacum,* revealed an abundance of the characteristically blunt rounded tips of the zoögloeae of the parasite arrested in place just as they pushed their way through the irregularly-shaped intercellular spaces of the mesophyll (Fig. 1, A, B; Fig. 2, C. D. E). The zoögloeae consist of bacteria surrounded by a matrix easily dif-

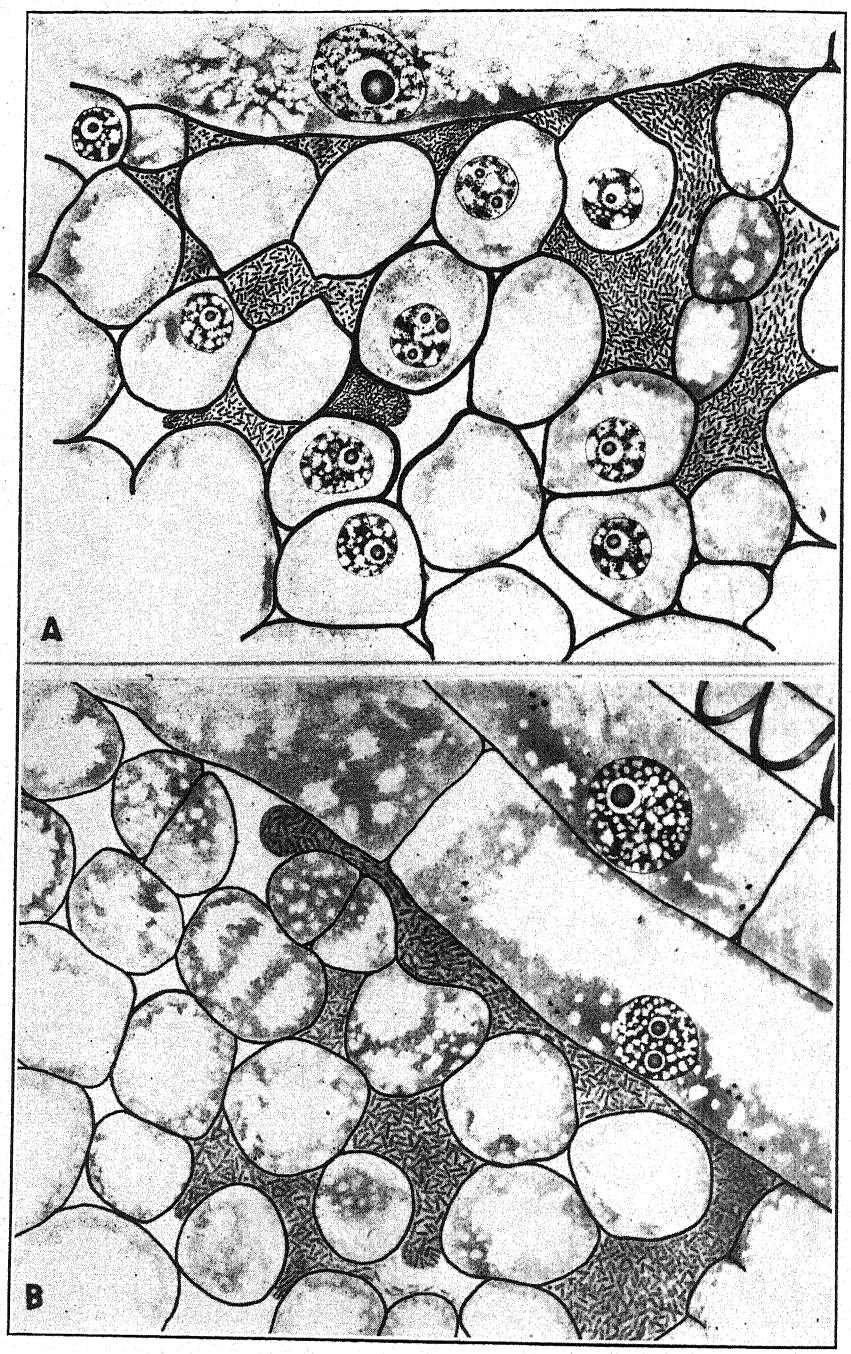


FIG. 1. A. Section of a leaf of the tobacco *Nicotiana tabacum* cut parallel with the epidermal layers, showing the distribution of the bacteria (*Bacterium tabacum*) as they invade the healthy tissues following inoculation. The region is that of the mesophyll adjacent to a small vascular bundle an elongated cell of which is shown above. At the lower left attention is directed to the two rounded tips of the zoögloeae. About 1250×.

B. Section cut through the same general region as that shown in A. One of the zoögloeae of *Bacterium tabacum* is shown advancing through the irregular intercellular spaces which lie adjacent to and parallel with a vascular bundle whose cells are shown in the upper right. The tip of this zoögloea is enlarged and rounded as it has advanced into a relatively large intercellular space. Three characteristically blunt and rounded zoögloeal tips are shown in the lower left and lower center of the figure. About 1250×.

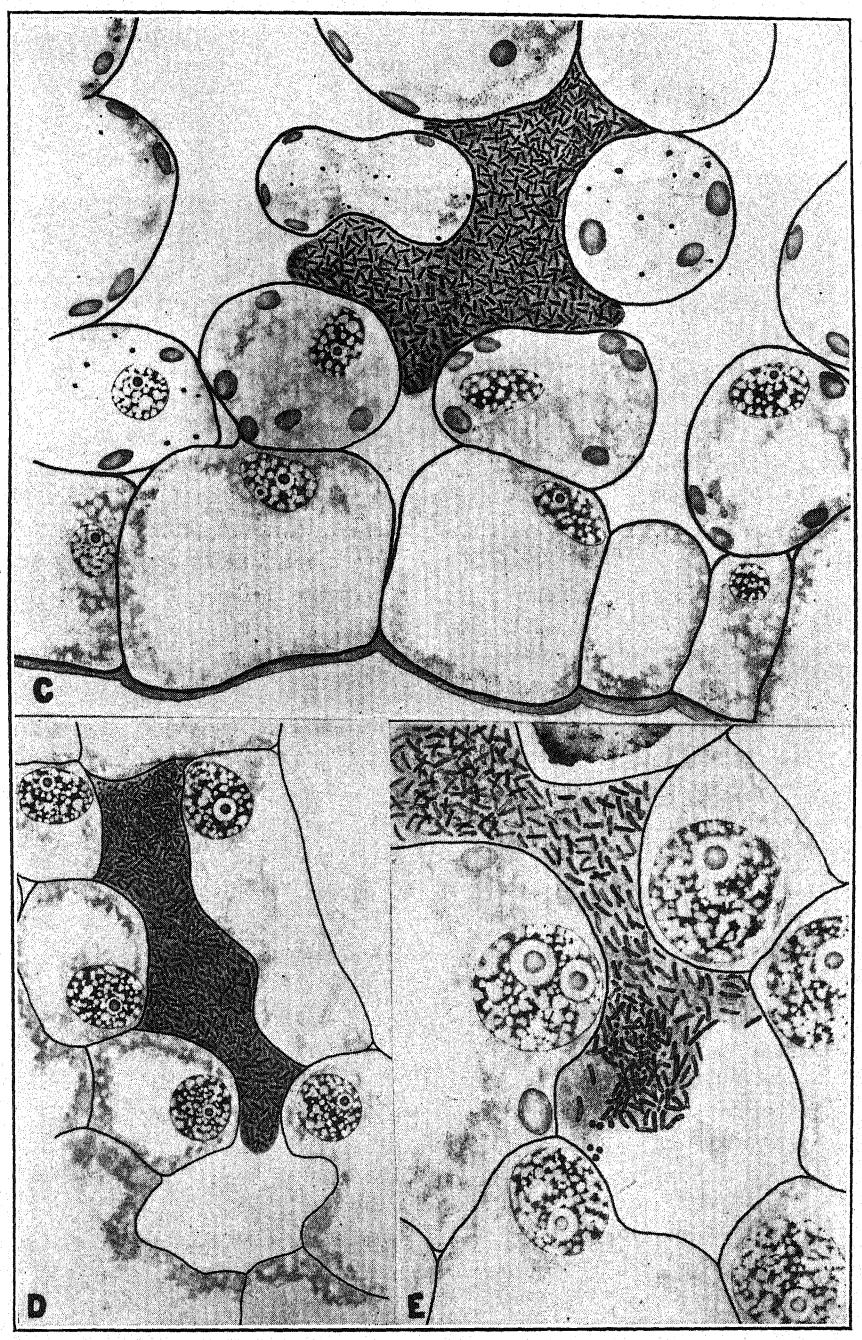


FIG. 2. Various forms of zoögloeae of *Bacterium tabacum* within the irregular intercellular spaces of the mesophyll of the leaf.

C. A transverse section of the leaf of a tobacco seedling naturally infected with "Wild Fire." The initial infection had apparently occurred on the upper surface of the leaf. The bacteria had migrated outward and downward through the intercellular spaces of the palisade layer, reaching the lower mesophyll where the zoögloea is shown. The zoögloea has assumed the irregular shape of the intercellular spaces of this region. Four blunt, rounded zoögloeal tips indicate the directions of migration of the bacteria. The cells of the mesophyll have nuclei cytoplasm and chloroplasts. The lower epidermal cells without chloroplasts are shown below. About 1250 ×.

D. A section adjacent to a vascular bundle of the leaf with an elongated zoögloea occupying a short narrow intercellular space parallel to the cells of the bundle. The rounded tip is shown about to enter a much larger intercellular opening. About 1250 ×.

E. A section through the mesophyll of the leaf showing a zoögloea advancing into a relatively large intercellular space. The zoögloea has three advancing tips. About 2500 ×.

ferentiated by Flemming's triple stain. In most instances the bacteria are stained red or purple, while the matrix shows a greater affinity for the orange G. The forms assumed by the zoögloeae indicate that the matrix is of a semifluid or jelly-like consistency and easily capable of a flowing movement. Apparently, they do not readily adhere to the walls surrounding the intercellular spaces which they occupy. In this respect the behavior of the zoögloeal mass is similar to that of a heavy fluid in a tube, the blunt rounded tips of the zoögloeae resembling the characteristic convex meniscus. Of interest also is the orientation of the bacteria with their long axes in the direction of the advancing tip which can frequently be observed where the zoögloea had pushed into a very narrow intercellular space (Fig. 1, B). Bacteria are more abundant in the older parts of the zoögloeae and fewer in the advancing tips (Fig. 2, E). In the older parts of the zoögloeae the bacteria become so crowded and stain so deeply that the matrix is often entirely masked.

Probably the most conspicuous feature of the zoögloeae of *Bacterium tabacum* in the tissues of the tobacco leaf is the presence of a large number of the rounded, blunt, advancing tips (Fig. 1, B; Fig. 2, C). A condition similar to that illustrated in figure 1, B, is very frequently found. This phenomenon is probably associated with the labyrinthine intercellular spaces of the lower mesophyll of the leaf in which the organism is found and is merely its response to its opportunity for simultaneous growth in many directions.

In the invasion of the large irregularly shaped intercellular spaces, the zoögloeae assumes the shapes of the cavities they chance to occupy (Fig. 1, B; Fig. 2, C, D, E), so that an outstanding feature of the zoögloeae is the variety of forms which are found in the lower mesophyll tissues of the leaf. An unusual form of zoögloeal tip was seen where the structure had pushed out into a relatively large intercellular space. Under such conditions the advancing face of the zoögloea became irregular (Fig. 2, E) and frequently had several advancing points.

COMPARISON OF BACTERIUM TABACUM WITH CERTAIN OTHER SPECIES OF BACTERIAL PHYTOPATHOGENES

Some very instructive comparisons may be made among those species of bacterial plant parasites the migration of which has been described. The most interesting features for comparison are the nature and form of the zoögloeae and the relative rates of migration of the bacteria through the tissues of the host. While there are many unsolved problems associated with the bacterial phytopathogenes, the features in which the published reports show greatest accord are the observations relating to the

physical properties of the zoögloeae, their responses to their environment in assuming the shapes of the intercellular spaces of the host tissues, and the relation of these facts to the general method of migration of those bacterial phytopathogenes which have been adequately investigated in regard to this feature of their behavior.

The observation of *Bacterium tabacum* in the tissues of the tobacco leaf leads to the conclusion that its behavior is similar in all essential features to that of the species of bacterial plant parasites recently described. This organism migrates in the form of zoögloeae which, at least in the early stages of the infection, are confined to the intercellular spaces of its host. The lumina of cells mechanically injured may also be occupied by the organism. The zoögloeae assume the shapes of the irregular spaces of the mesophyll and are, therefore, of so many forms that no one form may be considered as typical. The conception of the physical nature of the zoögloeae of *Bact. tabacum* based on their microscopic appearance agrees in all essential respects with that expressed by the recent workers (*loc. cit.*) concerning the zoögloeal structures in the species they studied; that is, the zoögloeae of *Bact. tabacum* consists of bacteria surrounded by an apparently gelatinous semifluid matrix and pass into the intercellular spaces of the tissues, where they assume the shapes of the cavities they occupy.

Though the study of the rates of migration of parasitic organisms is interesting, their accurate determination is beset with considerable difficulty. It must be realized that various inconstant environmental factors, such as temperature, moisture, and the condition of the host, must be considered; since these affect the rate of growth, they may also be expected to affect the rate of migration, which is probably a growth phenomenon. Differences in rates of migration may also be expected to characterize distinct strains of parasitic organisms. The rate of migration determined for one strain of an organism, therefore, may not be the rate which another strain of the same species may show. It is well known that the species of bacteria vary in many characteristics, such as the length of incubation period and the nature of the symptoms of the diseases which they produce. In the light of such knowledge, it may be expected that the various species of bacteria will show variation also in their rates of migration.

Bacterium tabacum migrates very slowly. It is extremely difficult to measure accurately the rate of migration of this parasite, since mesophyll tissue offers only a very crooked and irregular path. The fact that in this species advancing zoögloeae were found only in material fixed 48 to 72 hours after inoculation may be regarded as an indication of a slower rate of migration as compared to that of *Bact. tumefaciens* in which migration is well under way within an hour following inoculation (10).

SUMMARY

The causal organism of wild fire of tobacco, *Bacterium tabacum*, migrates in the form of zoögloeae in a manner comparable in all essentials with those species of parasitic organisms the methods of migration of which have been recently described.

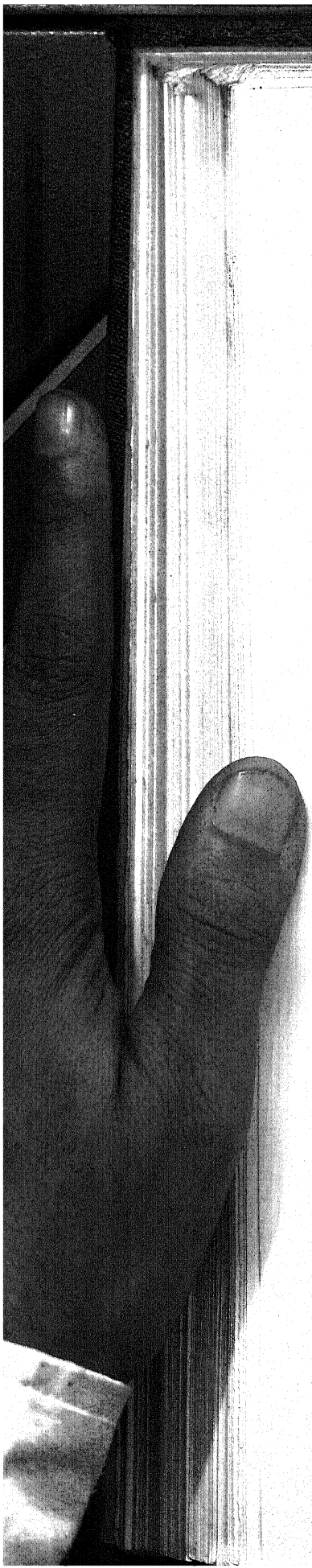
The rate of migration of *Bact. tabacum* is very slow as compared with that of *Bact. tumefaciens*.

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BOOK REVIEWS

Comparative Morphology of Fungi. By Ernst Albert Gäumann. Translated and revised by Carroll William Dodge. McGraw-Hill Book Company, New York, 1928. Gäumann's "Vergleichende Morphologie der Pilze" was a noteworthy contribution to mycological literature in bringing together the advances of forty years in a field of fertile research. The announcement of an English translation was therefore most welcome.

Dodge's work is a revised translation. The sequence of the orders of the Basidiomycetes has been rearranged in accordance with Gäumann's suggestions in the original book. More or less extensive revisions have been made in the treatment of the Tuberales, the Laboulbeniales, the Polyporales, and the Gasteromycetes. Minor changes occur throughout the book. One outstanding feature of the whole work is that Dodge has taken the liberty to change a sentence, a paragraph, or a section without indicating that it is different from the original except by stating in a most general way in his preface to the American edition that he has rewritten certain sections and corrected ambiguities in the original text. In a translation, an accurate rendition of the original author's ideas is expected of the translator and it would seem best that differences of opinion or corrections be given in authenticated footnotes. As it stands the book can scarcely be called Dodge's; neither does it accurately represent the original Gäumann.

Translation of so extensive a work is a laborious and tedious task and any one undertaking it deserves much commendation. It is greatly to be regretted that more time was not taken for correction of typographical errors and for critical reading. When this sentence, "Diese Auffassungen bieten (unter der Voraussetzung, dasz die Protobasidie wirklich eine ursprüngliche Form der Basidie ist) den Vorteil, dasz sie in den Uredineen das gesuchte Verbindungsglied zwischen den Ascomyceten und den Basidiomyceten schaffen: letztere hätten sich aus ersteren über uredineenähnliche, saprophytische lebende Formen entwickelt" (page 452) is rendered thus, "These conceptions (assuming that the phragmobasidium is originally a form of basidium) offer the advantage that they create in the Uredinales the sought-for link between the Ascomycetes and the Basidiomycetes; the latter had developed from the former through the rust series to the saprophytic forms" (page 587) one loses confidence in the accuracy of the English he is reading.

Frequent mistranslation of words, typographical errors, and ambiguous sentences which lose their meaning by too literal translation and omission of essential words or phrases make an understanding of some parts of the

text very difficult. Examples of mistranslations are "flagsmut" for "Flugbrand" (page 596), "next" for "zunächst" (page 52), "shortly before" for "kurz nach" (page 81), "swelling sporangiospore" for "Sporangiospore bei schwächerer Vergröszerung" (page 96), "fuse" for "vermengen" (page 274). Typographical errors are more serious and numerous than one would expect in a work of this sort; for example, "sexual" instead of "asexual" (page 117), "conidia" instead of "coremia" (page 177), "aplanogametes" instead of "planogametes" (page 17), "zoospores" instead of "oospores" (page 62). The chapter on the Laboulbeniales, where the author has wholly abandoned the language of the original and has rewritten the text in a clear and forcible manner, stands in sharp contrast to the other chapters which are presumably free translations. There is a disconcerting lack of consistency in carrying through the whole work the author's general theme of the development of the dicaryophase in the fungi, where Dodge inserts without comment the work of certain authors not treated by Gäumann; for example, Elliott's account of the nuclear behavior in *Ceratostomella fimbriata* (page 262). The interpolations of the translator are not always accurate: for instance, attributing to Sadebeck the separation into Exoacus of the species of the Exoascaceae with 4- to 8-spore asci and into Taphrina of those in which the spores bud in the ascus (page 164).

Gäumann's work has been one of the outstanding contributions in the field of modern mycology. He has assembled a vast amount of mycological data and presented them with remarkable clarity in an orderly sequence. He has made valuable contributions on the significance of the dicaryophase as a distinguishing characteristic in the phylogeny of the fungi. If Dodge's revised translation will serve to bring this contribution more generally to the knowledge of American readers it will serve a useful purpose.—LOUISE DOSDALL, University Farm, St. Paul, Minn.

A Monographic Study of Sweet-Potato Diseases and Their Control. By L. L. Harter and J. L. Weimer. United States Department of Agriculture Technical Bulletin 99, 117 pages, 24 figures, 26 plates. Washington, D. C. 1929.—In recent years the ever-increasing tendency toward crop specialization among plant pathologists has become more and more pronounced. Coincident with and as a natural outcome of this development, the publications on a given subject have become increasingly scattered, thus working hardships upon the specialist whose library facilities are limited. The obvious solution of the difficulty is the publication of monographic studies of the diseases of individual crops by those whose personal investigational work and library facilities qualify them as specialists.

Inasmuch as phytopathology in its broadest aspects knows no political

boundaries, it is quite fitting and proper that the United States Department of Agriculture should take the initial step in the issuance of individual crop-disease monographs. In presenting to plant pathologists "A Monographic Study of Sweet-Potato Diseases and Their Control," by L. L. Harter and J. L. Weimer, the Federal Government has recognized a need and has taken such measures as fall within its province to mitigate the bibliographic conditions of less fortunate plant pathologists.

Harter and Weimer, as a result of over sixteen years of research devoted mainly to sweet-potato diseases, are admirably qualified for the responsibility of monographing the diseases of this crop, the second most important vegetable in the United States. They have brought together within the confines of 117 pages all the important available data on the diseases of sweet potatoes—now spread in the literature of over twenty countries and in more than seven languages—and have condensed and correlated certain heretofore unpublished data of their own.

The major divisions of the bulletin are made on the basis of the causal agency involved—fungi, nematodes, and physiological diseases or those of unknown cause—and their control. Each of these is further subdivided into important and minor diseases of field and storage.

Each disease is exhaustively treated in much the same general manner. The history of the malady, its geographical distribution and economic importance, the symptoms, and the causal organism are fully described. If the cause is not known, a discussion is given of the factors contributing to the presence of the disease and their possible etiological relation. Of special interest—and one of the most valuable portions of the bulletin—are the treatments of the causal organisms involved in the several diseases. These are thoroughly discussed from the standpoints of historical taxonomy, pathogenicity, morphology, life history, and dissemination.

Where a disease is of importance in both field and storage, the former phase has received the principal discussion and is only sufficiently reviewed with reference to the storage aspect to orientate the disease and its causal organism in the reader's mind.

The discussion of the control measures has been left for a major section, since the same methods or modifications of them are applicable to all. The control of the field diseases of the sweet potato is recommended through exclusion of diseased seed stock from foreign countries by means of adequate quarantine regulations, through inspection and certification of domestic seed and plants, through the use of disease-resistant varieties in so far as such may exist or may in the future be developed, and through eradication by following approved agricultural practices and seed treatment. Elimination of disease through thorough inspection of the harvested crop and the selection of disease-free stock for seed purposes, the proper methods

of digging and handling, the correct management of the storage house and the stored crop, and the fumigation—ill-advised at best—of sweet potatoes in storage are covered in the discussion of control of storage diseases.

Among the new or little-known diseases covered in the monograph, the following may be mentioned: Mottle necrosis, caused by *Pythium ultimum* and *P. scleroteichum*; rootlet rot, caused by *P. ultimum*; a leaf spot caused by *Septoria bataticola*; a leaf spot caused by a species of Alternaria; rust, caused by *Coleosporium ipomoeae*, with an account of its parasite, *Monosporium uredinicolum*; leaf spot, caused by *Cercospora batatae*; damping off, attributed to a species of Sclerotinia similar to *S. minor*; a foot rot caused by *Macrosporium solani*; growth cracking; fasciation; intumescence; sunscald; minor storage diseases caused by *Mucor racemosus, Alternaria* sp., *Penicillium* sp., *Botrytis cinerea, Epicoccum* sp., *Gibberella saubinetii*; *Fusarium culmorum, F. acuminatum, Trichoderma köningi*, and *Sclerotinia* sp.; and minor reports on about twenty other fungi which have, in the course of years, been isolated from sweet potatoes.

The unusually clear and well-selected illustrations, of which there are fifty—24 text figures and 26 plates—,show the smallest characters of the many diseases.

A bibliography of 211 titles, including all the important known publications on sweet-potato diseases, as well as many supporting papers, is appended.

In this monographic study of the diseases of a single crop, the authors have done a commendable piece of work, enormous in scope, exhaustive in treatment, and—what is most important—simply, though none the less scientifically, written. It is to be hoped that similar monographs will be forthcoming for other crops and that, later, following the accumulation of new data, revisions will be made so that the current usefulness of the works may not be lost through becoming out of date.—W. A. WHITNEY, Washington, D. C.

PHYTOPATHOLOGY

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STUDIES IN THE GENUS PHYTOPHTHORA IN MYSORE

I. Heterothallic Strains of Phytophthora

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INTRODUCTION

Sexuality in the genus Phytophthora is a subject which has recently attracted considerable attention. As is well known, in certain species such as, for example, *Phytophthora infestans* (Mont.) de Bary and *P. arecae* (Coleman) Pethyb., oospore formation is a rare occurrence in pure culture and it has not yet been observed in nature. Other species, on the contrary, such as *P. phaseoli* Thaxt., form oospores readily both in nature and in pure culture, while in a few, such as *P. erythroseptica* Pethyb., oospores seem to be the predominant reproductive body when the organisms are grown in pure culture.

The conditions under which oospores develop in *Phytophthora* spp. have not been fully determined. Clinton (5) sought first to prove that oospore production was dependent on the presence of male and female mycelial strains. With this object, he attempted to obtain single-spore cultures of *P. phaseoli* by plating melted agar containing the spore-bearing mycelium shaken through it. Since the resulting colonies ran into one another, Clinton could not be sure that he was dealing with single-spore cultures. When grown on favorable media, these individual colonies developed oospores, but whether this was due to homothallism or to the mixing of mycelia from two colonies he was not sure. Clinton (6) was able to obtain oospores by growing *P. infestans* and *P. phaseoli* together. Gadd (9) obtained a number of strains of *P. faberi* Maub. occurring in Ceylon on various plants such as cacao (pods), papaya (fruit and stem), Hevea (pods), *Artocarpus incisa* L. (fruit), *Dendrobium MacCarthiae* Thw., and *Odontadenia speciosa* Benth. All the cultures were started from single sporangia. All possible combinations of two strains were grown on agar slants. In general, oospores were produced when any of the first three strains (cacao group) were grown

[1] The writer wishes to acknowledge his indebtedness to Dr. Leslie C. Coleman, Director of Agriculture in Mysore, for the encouragement and helpful suggestions given in the course of the investigation.

with any of the other four (rubber group). Any two strains of the same group did not form oospores when grown together. This separation into two groups showed, however, some exceptions. According to Gadd, the Dendrobium strain never formed oospores with the two papaya strains, nor did the bread fruit strain with that obtained from papaya fruit.

Lester-Smith (10), working along similar lines, obtained oospores in cultures of cacao, West Indian cocoanut, and cotton strains, when each was grown with *P. parasitica* Dastur. Ashby (1) found that large oospores were formed when *P. cinnamoni* Rands was grown with *P. parasitica* and with strains of *P. cryptogaea* Pethyb. & Laf. Recently, Ashby (2), working with a large number of strains of *P. palmivora* Butl. (*P. faberi* Maubl.), adopted Gadd's grouping, viz, "cacao" and "rubber" strains. Nine strains of the cacao group, when grown in paired cultures with fourteen strains of the rubber group, were found to produce oospores, the exceptional case being that the Citrus strain from the Philippines, when paired with the cocoanut strain from India, did not produce any oospores, although these strains belonged to two different groups.

The present investigation was originally undertaken with the object of ascertaining what relationships, if any, exist between the various forms which the author has been able to collect in Mysore. During the course of this investigation interesting observations have been made on the sexuality of these forms, the preliminary results of which form the subject of this paper.

PHYTOPHTHORA STRAINS OCCURRING IN MYSORE

A brief report of the various Phytophthora strains occurring in the area where the ravages of *P. arecae* on arecanut are rampant has already been given by the writer (12). A short description of these strains and the symptoms produced by them follows.

1. *Phytophthora on Santalum album* L. Shedding of the leaves, which show brown patches, is a prominent feature of the disease produced by this fungus. The tender shoots, flowers, and fruits are all affected. Sporangia: 41 μ × 25.9 μ. Sexual bodies not found in nature nor in cultures.

2. *Phytophthora on Loranthus longiflorus* Desr. Parasitic on *Mangifera indica* L., *Eugenia jambolana* Lam., etc. This is also of very wide occurrence. Affected leaves turn rusty brown. Sporangia: 42.5 μ × 32.8 μ. No oospores found either in nature or in cultures.

3. *Phytophthora on Jatropha curcas* L. The disease appears as brown patches on the fruit which later turns black. This is the commonest form in the area as these plants serve as hedges for paddy fields. Sporangia: 37.8 μ × 26.7 μ. Chlamydospores (28 – 29 μ) are found in large numbers on

the fruits and in cultures. In oat-agar cultures the aerial mycelium practically fills the tube. The fungus answers to the description of *P. jatrophae* Jens. (*P. faberi* type.) No oospores found in nature nor in cultures.

4. *Phytophthora on Bryophyllum calycinum* Salisb. The disease appears as round patches on the leaves, often spreading in concentric rings Sporangia: 42.5 μ × 26.2 μ. Oospores occur both on fallen rotting leaves and in cultures.

5. *Phytophthora on Artocarpus integrifolia* L. Almost every tree in the area is affected by this disease which causes the shedding of leaves and rotting of the tender inflorescences. Sporangia small, round. Oospores found in abundance on the affected inflorescences. In oat-agar cultures the aerial mycelium is not at all prominent, only oospores and no sporangia being formed. Sporangia are, however, formed if the fungus is grown on sterilized flies in distilled water, as in the case of *P. erythroseptica* Pethyb.

6. *Phytophthora on Colocasia antiquorum* Schott. Probably *P. colocasiae* Rac. Oospores not found in nature and rarely in oat-agar cultures.

7. *Phytophthora on Ficus hispida* L. (*Wild Fig*). This strain is not of very wide occurrence. Only the fruits are affected and these drop. Sporangia ellipsoid, with prominent crown-like papillae. Oospores found in large numbers on the fruits and in cultures.

OOSPORE PRODUCTION IN THE ARECA, SANTALUM, LORANTHUS, AND JATROPHA STRAINS

These first four strains of Phytophthora, viz, *P. arecae* and the Santalum, Loranthus, and *Jatropha curcas* strains, form oospores rarely or not at all in nature or in culture and were selected for further study. Neither Coleman (7) nor the writer has ever observed oospores of *P. arecae* in nature or in oat-agar cultures. They were obtained by Coleman in a set of cultures, wherein fresh aseptically-taken arecanuts were placed in Roux tubes and inoculated with mycelium of *P. arecae*. It is not unlikely that material from these particular cultures was sent to Rosenbaum (13) who reported the presence of oospores in his cultures of *P. arecae*. A possible explanation for this phenomenon is indicated in the course of this paper. The writer has examined many affected arecanuts and oat-agar cultures but has never observed oospores at any time. For the present and pending further work, *P. arecae* is taken as a nonoospore-forming strain. Preliminary trials in which the mycelia of *P. arecae* and the Santalum Phytophthora were grown together on oat-agar were made with the result that oospores formed where the mycelia met. With the idea of making a careful study of this phenomenon, single-spore cultures of the four Phytophthora strains were made.

Malt-agar tubes inoculated with zoospore suspensions were poured into plates and the resulting growths from single spores were picked with the aid of a binocular microscope. Zoospores for these cultures were obtained by placing in sterilized water bits of mycelium from different tube cultures so as to ensure a fairly representative sample. Twenty-five of such single-spore cultures of each of the four Phytophthora strains under consideration were started in oat-agar tubes. It seemed certain that single-zoospore cultures formed a surer basis for work than the single-sporangium cultures of other workers, as the sporangium may contain a number of zoospores with possibly different genetic constitution.

All possible combinations of two of these various strains were grown together. At first the two bits of mycelium from single-spore cultures were grown in roll-tube cultures and examined under the microscope. Although the danger of contamination is minimized by this method, it has the disadvantage that the thin film of the medium dries up after a time. Later cultures were all made in small Petri dishes on oat-agar or French-bean agar.

Single-spore cultures of the Areca strain when grown with those of the Santalum strain always produced oospores. If a single-spore culture of *P. arecae* and one of the Santalum strain are sown an inch apart on French-bean agar, the beginning of oospore formation may be observed on the third day at the junction of the two mycelia, while on the fourth or fifth day, a brownish line indicates the area of oospore production (Fig. 1, A). It is interesting to compare this and subsequent figures with those of Blakeslee (3) for plus and minus strains of Mucor and those of Claussen (4) for the heterothallic strains of *Pericystis alvei* A. D. Betts, which causes a disease of bees known as "Kalkbrut." Under similar conditions, oospore formation, accompanied by the development of a brown line, was observed when

TABLE 1.—*Oospore-formation reaction resulting from joint culture of differing strains of Phytophthora on oat-meal and French-bean agar*

Strains grown together		Oospore formation	Oospore size
Areca	13[2] × S 11	+	30–31 μ
"	13 × L 11	–	
"	13 × J 12	+	26–27 μ
"	13 × A 11	–	
Santalum	11 × L 11	+	30–31 μ
"	11 × J 12	–	
"	11 × S 14	–	
Loranthus	11 × J 12	+	29–30 μ
"	11 × L 14	–	
Jatropha	6 × J 12	–	

[2] Numbers indicate single-spore strains.

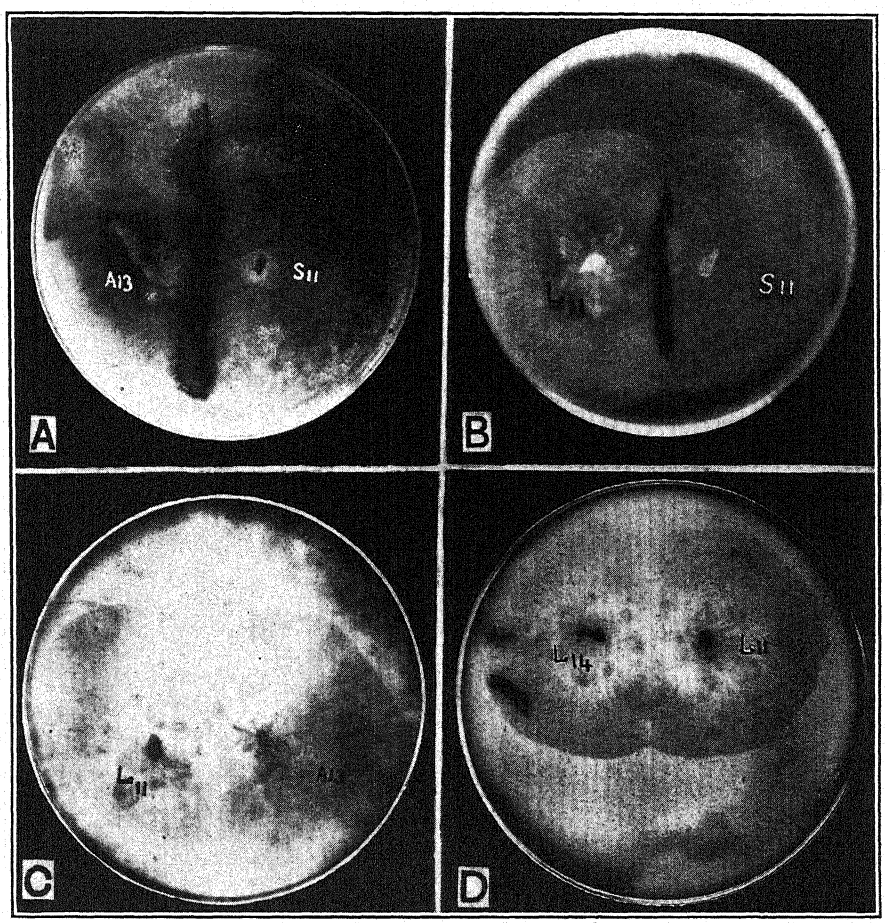


FIG. 1, A. Paired single-spore culture of the Areca (A. 13) and Santalum (S. 11) strains. Dark line in the middle indicates area of oospore formation. Photograph taken nine days after starting culture. B. Paired single-spore culture of Loranthus (L. 11) and Santalum (S. 11) strains, showing dark line at the juncture of the two mycelia Photograph taken five days after starting culture. C. Paired single-spore culture, six days old, of the Areca (A. 13) and the Loranthus (L. 11) strains. No formation of dark line. D. Paired single-spore culture of two Loranthus strains. No formation of dark line. All photographs, except B, taken with illumination from below.

single-spore cultures of the Loranthus and Santalum strains (Fig. 1, B), the Loranthus and Jatropha strains (Fig. 2, B), and the Areca and Jatropha strains (Fig. 2, C) were grown together.

In no case was oospore formation observed when single-spore cultures of the Areca and Loranthus strains (Fig. 1, C) or the Santalum and Jatropha strains (Fig. 2, A) were grown together.

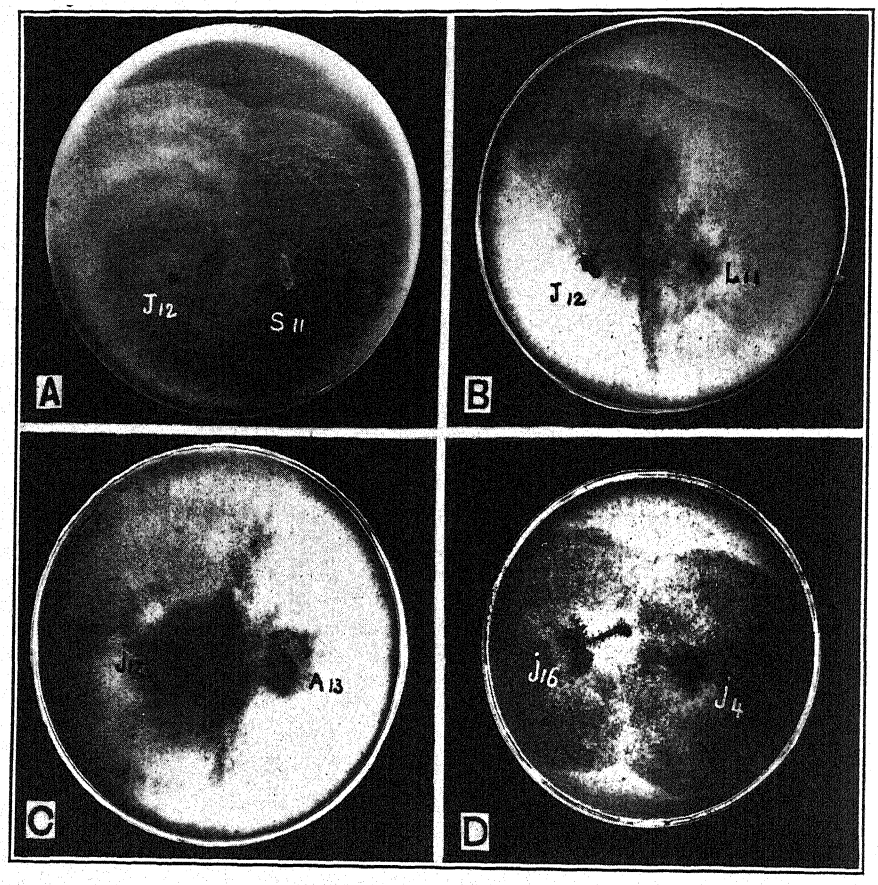


FIG. 2, A. Paired single-spore culture of the Santalum (S. 11) and Jatropha (J. 12) strains of Phytophthora. Culture six days old. No formation of dark line. B. Paired single-spore culture, five days old, of the Loranthus (L. 11) and Jatropha (J. 12) strains of Phytophthora, showing dark line. C. Paired single-spore cultures of the Areca and Jatropha strains of Phytophthora, showing dark line in the middle. D. Two paired single-spore Jatropha strains. Substage illumination for all photographs except A.

PROOF THAT ARECA STRAIN PRODUCES ANTHERIDIA; SANTALUM STRAIN, OOGONIA

In studying the details of the oospores, staining with Sudan III according to Strasburger's method was found to give excellent results. When mounted in glycerine gelatine, the oospores retained the stain for about two months. Löhnis (11) pointed out that Sudan III could be used as a differentiating stain for the mycelium of Phytophthora in tissues, while other fungi do not take this stain. The oospores were always of the amphigynous

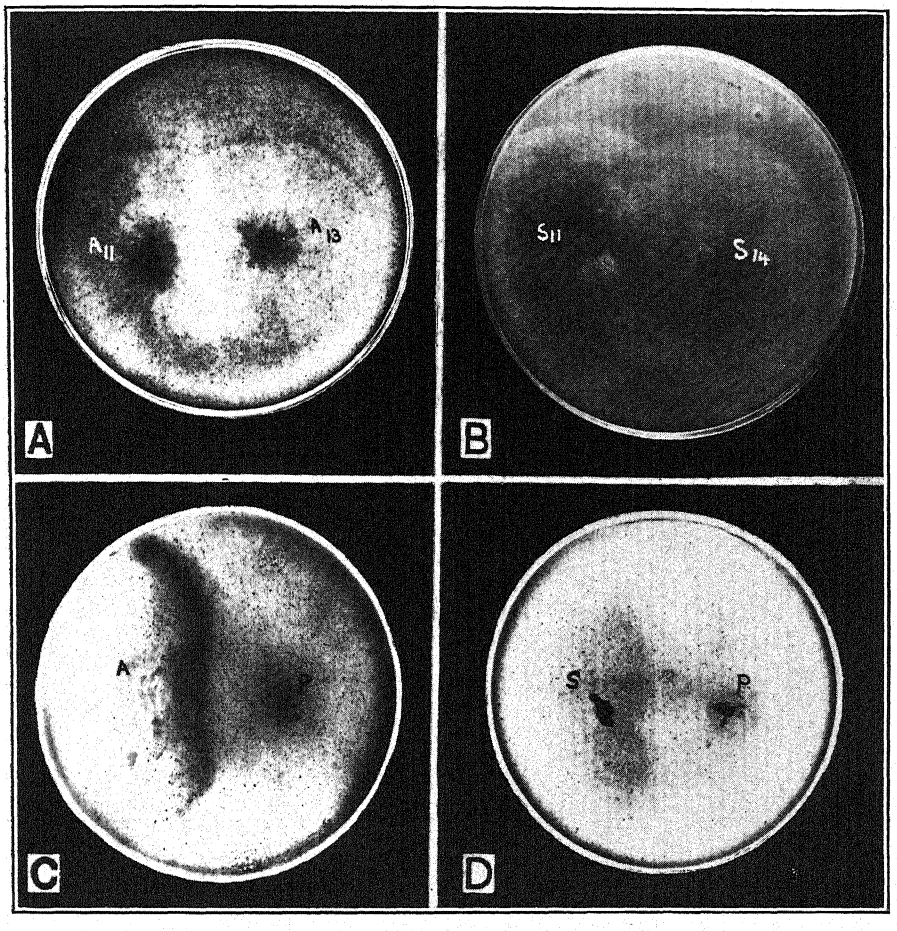


FIG. 3, A. Culture of two single-spore strains of P. arecae. B. Culture of two single-spore Santalum strains. No formation of dark line in A and B. C. Paired single-spore culture of the Areca and parasitica strains. Formation of dark line is prominent. Culture six days old. D. Paired single-spore culture of the Santalum and parasitica strains. No formation of dark line. Substage illumination for photographs except B.

type, the antheridia and oogonia being borne on different thalli. Since the oospores are formed at a place where the two thalli interlace, forming a network, great difficulty was experienced in determining which strain bears the antheridium and which the oogonium. This difficulty was partly overcome by checking the rapid advance of the two colonies by the following method:

Two strips of mica with tiny holes were so placed vertically at the bottom of the Petri dish as to intervene between the two spore sowings. It was found that the mycelium which grew through the holes in this barrier was

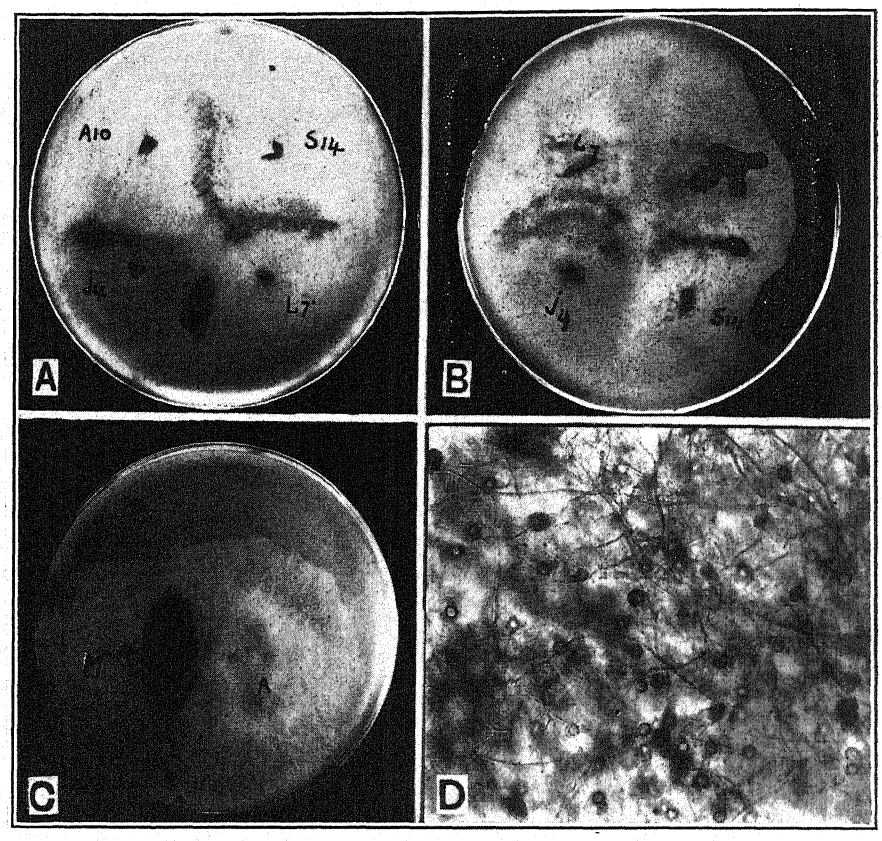


FIG. 4, A. Single-spore cultures of the Areca (A. 10), Santalum (S. 14), Loranthus (L. 7), and Jatropha (J. 4) strains so placed that a dark line formed at the place of union of each pair. B. Culture of the same four strains, arranged differently to show the formation of dark bands between strains of opposite sex only. No formation of dark line, either between the two male strains (above) or the two female strains (below). C. Paired culture of single-spore strain of *P. arecae* and *P. meadii*. Formation of dark patch in the middle can be seen. D. A portion of the dark line shown in figure 1, A, low-power magnification, showing numerous oospores. Photographs illuminated from below except in C.

sparse enough to facilitate observation. When the Areca and Santalum strains were thus grown together, it was observed that the antheridia were borne on the mycelium of the former and the oogonial incepts could be traced to the mycelium of the latter (Fig. 5, A).

Up to the present, the work done has related to four strains of Phytophthora which normally do not produce oospores either in nature or in cultures. The exceptional case wherein oospores were reported in *P. arecae*

by Coleman (7) may perhaps be explained by assuming that the Areca and Santalum strains have by some chance found their way into the culture. If we take into consideration the fact that these two strains of Phytophthora are found in close proximity in nature, breaking out almost at the same time, such a possibility is not excluded. How far such a mingling of these two strains occurs in nature is yet to be determined.

OOSPORE PRODUCTION IN PHYTOPHTHORA PARASITICA AND P. MEADII

Through the kindness of Mr. W. McRae, Imperial Mycologist, Research Institute, Pusa, cultures of *P. parasitica* and *P. meadii* were obtained. Though oospores had been reported previously in these two Phytophthoras by Dastur (8) and McRae, respectively, no oospores developed either in the tube cultures originally received or in our subcultures. Probably these cultures had lost the power of forming oospores, as has been reported in other species of Phytophthora by various workers.

TABLE 2.—*Showing results obtained by pairing P. parasitica and P. meadii with the Areca, Loranthus, Santalum, and Jatropha strains*

Strains grown together			Oospore formation
Areca	×	parasitica	+
Loranthus	×	parasitica	+
Santalum	×	parasitica	–
Jatropha	×	parasitica	–
Areca	×	meadii	+
Loranthus	×	meadii	+
Santalum	×	meadii	–
Jatropha	×	meadii	–

It therefore appears that *Phytophthora parasitica* and *P. meadii* resemble the Santalum strain in that they form oospores when paired with either the Areca or the Loranthus strain.

DISCUSSION OF RESULTS

The results given above seem to indicate quite clearly that heterothallism exists in some species of Phytophthora. Before attempting to evaluate the results obtained, it is necessary to consider whether, in an oospore-forming or apparently homothallic species, two sexual strains, male and female, actually exist. Clinton (5) attempted to separate two such strains in the case of *P. phaseoli,* grown in pure culture, but was not able to obtain definite results. Whether homothallism exists in certain species here under investigation is a question now being studied.

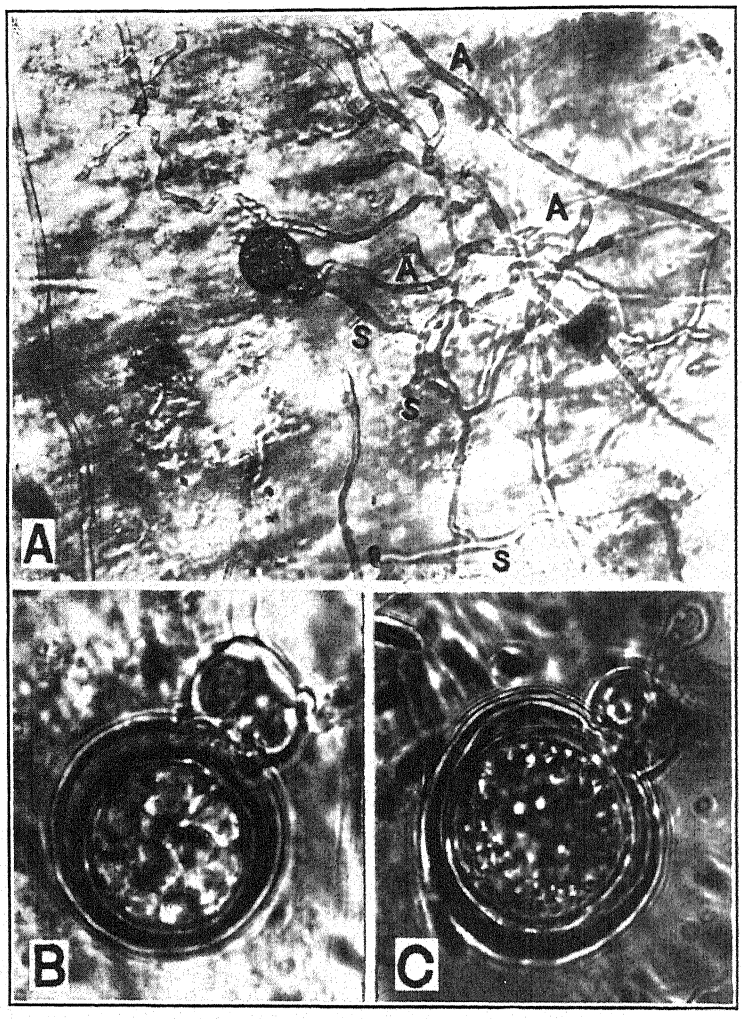


FIG. 5, A. Photomicrograph showing the mycelial relationship in formation of an oospore in a paired culture of the Areca and Santalum strains. (A) Mycelium of the Areca strain attached to the antheridium and (S), the mycelium of the Santalum strain attached to the oogonial incept. Photograph taken directly from a Petri-dish culture. B. Photomicrograph of an oospore formed in a paired culture of the Areca and Santalum strains × 900. C. Photomicrograph of an oospore formed in a paired culture of the Areca and Jatropha strains × 900.

The behavior of the three strains, Areca, Loranthus, and Santalum, can be explained if we suppose that the first two are male and the third, female. Oospores always formed when the Santalum strain was grown with either of the other two, but under no conditions were they formed when the Areca

and Loranthus (male) strains were grown together. As stated above, the mycelium of the Areca strain has been shown to bear the antheridia when single-spore cultures of this strain are grown along with the Santalum strain.

Lester-Smith (10), working with cultures of various strains of *P. palmivora* paired with *P. parasitica*, held the view that a normal strain of *P. palmivora* was potentially bisexual and that, in mixed cultures, "a low rate of metabolism characterized by low water content and a different ratio of food materials" induced by the presence of a competing organism, resulted in the development of oospores. This view does not explain why oospores are produced only when certain strains are paired.

While it is probable that some change in the media takes place where two oospore-forming strains meet, as evidenced by the brown coloration, it cannot be definitely said whether this is due to some sort of staling substance or to the thick brown walls of the oospores. The results obtained, however, seem to show clearly that the formation of oospore is due not to any effect of growth on the medium but to the union of two strains of opposite sex.

That the Areca and Loranthus strains are male and the Santalum strain is female is supported by the results obtained from the crosses made with *P. parasitica* and *P. meadii*, in which oospores were formed only when these species were grown with the Areca and Loranthus strains and not with the Santalum and Jatropha strains. The cultures of *P. parasitica* and *P. meadii* used in this work probably contained only female strains of mycelia, but a careful microscopic examination of the cultures, such as has been carried out in the case of the Areca-Santalum combination, will have to be made before this question can be definitely settled. These two species, though originally bisexual and able to produce oospores, had lost their power of forming them when received from Pusa by the writer. This loss of the power to produce oospores in culture was observed by Clinton (5), Rosenbaum (13), and Dastur (8). Clinton was of the opinion that in cultures of *P. infestans* which failed to show oospores in culture the male mycelium had deteriorated, since the oogonia appeared more frequently than antheridia on oat-agar.

The interesting question remains as to whether, in the cases observed, we have to do with male and female strains of the same species or whether the oospores are the hybrid product of the union of male and female strains of different species. While the question requires very much more thorough investigation than has yet been possible, it seems probable that we have had both phenomena under observation.

The Santalum and Areca strains are morphologically almost indistinguishable. Their growth in culture media is different, however, the San-

talum strain being slower in growth and exhibiting much less aerial mycelium. The oospores resulting from a union of the two strains are of the same dimensions (30–31 μ) as those observed by Coleman (23–36 μ) and Rosenbaum (29.45 μ to 31.49 μ) for *P. arecae*.

If, as seems highly probable, either the male or female strain may disappear during culture on artificial media, leaving only the other, it would seem to be not too violent a stretch of the imagination to picture a similar occurrence on natural hosts so that, for example, we might have the male strain growing normally on Areca and the female strain on Santalum. These two host plants are found in sufficiently close proximity to allow for the occasional transfer of zoospores of one strain to the other host plant and mingling of mycelium in this host, thus accounting for the occasional production of oospores in the field and in culture, as noted by both Coleman and Rosenbaum. Experiments to test this possibility are at present under way.

In the case of oospore formation induced by the union of *P. arecae* with *P. parasitica*, it would appear more probable that we have to do with hybridization. The oospores in this case appear to be more or less intermediate in size (24–25 μ) between the oospores of *P. parasitica* and *P. arecae* as given in the literature. The further investigation of this interesting possibility through the germination of oospores and the study of subsequent generations will be taken up immediately.

The observations that have been presented in this paper are admittedly of a preliminary character. It has, however, been definitely established that the formation of oospores in paired cultures of two separate strains results from a union of the two strains which can be distinguished as male and female. In these cases oospore formation commences in three or four days. It has further been shown that where two male strains (Areca and Loranthus) are grown together no oospores are formed. Similarly, where two female strains (Santalum and Jatropha) are grown together no oospores are formed and in this case, we are apparently dealing with female strains of two different species. All the evidence so far collected supports Gadd's view that we are dealing with heterothallic forms of which the male and female strains have become more or less definitely isolated on different host plants. The possibility that absence of oospore formation in nature or loss of it in culture, so commonly met with in this genus, is due to the loss of one or other of the two sex strains and not to any external conditions merits serious investigation.

SUMMARY

In addition to *Phytophthora arecae*, seven strains of Phytophthora were collected in Mysore, occurring on *Santalum album* L., *Loranthus longiflorus*

Desv., *Jatropha curcas* L., *Bryophyllum calycinum* Salisb., *Artocarpus integrifolia* L., *Colocasia antiquorum* Schott, and *Ficus hispida* L.

Single-spore cultures of four of these strains, viz, those on Areca, Santalum, Loranthus, and Jatropha, were obtained and grown in paired cultures.

When single-spore cultures of the Areca or Loranthus strain were grown with any of those of the Santalum or Jatropha strain, oospores were formed at the juncture of the two mycelia, the area being indicated by the formation of a brown line.

Oospores did not develop when Areca and Loranthus strains or Santalum and Jatropha strains were grown together, nor was there any formation of the brown line.

When the Areca and Santalum strains were paired, the antheridia were traced microscopically to the mycelium of the Areca strain, the oogonia to that of the Santalum strain.

The view is held that oospore formation is the result of the fusion of heterothallic strains, the Areca and Loranthus strains having the male mycelia and the Santalum and Jatropha strains the female mycelia.

Cultures of *P. parasitica* and *P. meadii*, which had lost their capacity for oospore formation when grown with a male strain, such as *P. arecae*, readily formed oospores, but did not, when grown with a female strain, such as the one on Santalum. It is possible that the male strain in each of the cultures had been lost or had deteriorated.

The possibility of the two sexual strains becoming isolated on different host plants in nature is indicated as an explanation for the absence of oospores in some of the Phytophthoras.

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POTATO BLACKLEG: THE SURVIVAL OF THE PATHOGENE IN THE SOIL AND SOME FACTORS INFLUENCING INFECTION[1]

J. G. LEACH[2]

Although Appel (1) in his extensive investigations of potato blackleg presented evidence indicating that the pathogene could overwinter in the soil, many investigators in later years have reached opposite conclusions. Morse (12) after nine years of investigation, stated that "observations in Maine indicate that under the climatic conditions which exist there, infected seed potatoes are the sole source of infection and distribution and that the disease does not live over winter in the soil." Pethybridge (17), in 1912, expressed the opinion that the pathogene did not overwinter in the soil in Ireland. The sporadic appearances of the disease in the field and the abundant evidence that the pathogene is transmitted in seed tubers apparently have caused many people to assume, without sufficient evidence, that the pathogene does not survive in the soil.

The first attempts made in the United States to answer this question experimentally were made by Rosenbaum and Ramsey (19), who worked in Aroostook County, Maine, and at Norfolk, Virginia. They placed tubers thoroughly rotted by the blackleg pathogene in the soil in October. The following spring they planted disinfected tubers in the rows where the diseased tubers had been placed the previous fall. These tubers grew normally and no blackleg appeared. They were also unsuccessful in their efforts to reisolate the pathogene from the decayed tubers or from the soil about them. They stated that "In no case, therefore, was the blackleg organism found to live over a winter in the soil or in the tubers remaining in the soil."

In 1919 Ramsey (18) reported the results of additional experiments made in Aroostook County, Maine. Half-pint glass jars were filled with soil and 15 cc. of a virulent culture of *Bacillus atrosepticus* van Hall were poured into the top of each jar. One set of jars was overwintered out-of-doors, buried 5 inches in the ground; another set was kept in a cool base-

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[2] Some of the work reported in this paper was done while the author was a Fellow of the International Education Board, working in the laboratories of Dr. Sydney Paine, Imperial College of Science and Technology, London, England, and Dr. C. Stapp, Biologische Reichsanstalt, Berlin-Dahlem, Germany. The author expresses to them his appreciation for the facilities provided as well as for their helpful criticism and the many courtesies shown him.

ment. In the spring "dilution plates were made from the jars and all favorable looking colonies were tested for pathogenicity upon cut slices of potato tuber." None of the cultures isolated was found to be pathogenic.

Two additional experiments were made in which healthy tubers were planted in pots and watered frequently with broth cultures of the pathogene. All plants so treated remained healthy during the growing season, and at harvest time the tubers were free of rot. The author concluded that "These two experiments tend to show that unless the seed piece is infected at planting time, there is little chance that uninjured plants will contract the disease even though the causal organism is washed about the stem and root system."

Apparently, these negative results have been accepted as conclusive by most American workers in plant pathology. This is indicated by the frequency with which it is stated in extension literature that the pathogene does not survive in the soil and that infected seed stocks are the sole source of infection.

Stapp (21) has recently published the results of his extensive investigations on potato blackleg. He has demonstrated the ability of the pathogene to survive exposure to the winter conditions in Germany, both as a pure culture on agar and in the remains of infected tubers or vines buried in the soil. He did not, however, attempt to isolate the pathogene from the soil.

During the past five years considerable evidence has been obtained by the writer that the blackleg pathogene can and does survive in the soil under the severe winter weather conditions which characterize Minnesota. He has also obtained evidence to show that the negative results obtained by Rosenbaum and Ramsey (19) and Ramsey (18) do not in any way prove that the organism does not survive in the soil.

In 1926 (10) the writer described his efforts to produce the disease by seed-piece inoculation. A large number of tubers were artificially inoculated with a virulent culture of the pathogene and were incubated in a moist chamber until a considerable portion of each tuber had decayed. When these partly rotted tubers were planted in the soil under conditions favorable for germination, the decayed portions were walled off by a layer of wound cork and the disease developed no further. These experiments have been continued for the past three years with similar results. A few blackleg plants have appeared in the plots planted with such inoculated tubers, but the percentage has always been extremely small. In experiments extending over five years and including over 2,000 seed pieces, the increase in the amount of blackleg developing from inoculated tubers as compared with that developing in the check plots was less than one per cent.

When we consider the difficulty of producing the disease by the methods used in these experiments, it is obvious that the failure of the disease to

develop in the experiments of Rosenbaum and Ramsey (19) and Ramsey (18) does not prove that the bacteria are unable to survive in the soil. On the other hand, it shows that the bacteria, even if present in great abundance, are unable to infect an uninjured seed piece. The writer has repeated the experiments of Rosenbaum and Ramsey and has obtained similar negative results, but he is forced to interpret them differently.

Rosenbaum and Ramsey (19) and Ramsey (18) attempted to isolate the bacteria in the spring from soil heavily inoculated the previous fall but were unable to do so. The writer has repeated these experiments also, but, by using a suitable technique, it has been possible to isolate the blackleg pathogene not only from such inoculated soil but also from several samples of soil taken at random from potato fields in early spring before the appearance of the disease. Rosenbaum and Ramsey evidently poured dilution plates from the soil and attempted to isolate the organism by picking out "favorable looking colonies." When one considers the great number of bacteria which inhabit most soils and the similarity of their appearance on agar, it is not surprising that they did not succeed in reisolating the pathogene. Realizing this difficulty, the writer proceeded in the following manner. About one gram of the soil to be tested was thoroughly mixed with about 10 cc. of sterile beef-extract broth. This suspension was used for inoculating several surface-sterilized tubers by the "hole and plug" method previously described (10). After incubation in a moist chamber for four or five days a considerable amount of decay had developed. This decay was quite unlike that resulting from a pure culture of the blackleg pathogene and advanced much more slowly. It was usually accompanied by a foul odor and frequently gas production was evident. Most of the decayed tissue was next removed with a scalpel and a small portion at the margin nearest the sound tissue was removed with a sterile needle and used for inoculating a second lot of tubers or tuber slices. When these had started to decay, a third lot were inoculated in the same manner. Usually the virulence of the inoculum increased with each successive inoculation, and after three or four transfers the decay was quite typical of blackleg. When a small amount of freshly decayed tissue from the fourth or fifth inoculation was used for pouring separation plates, little difficulty was experienced in isolating a vigorously pathogenic culture similar in all respects to the culture used for inoculating the soil.

Patel (15), by using crystal violet bile agar as a selective medium, has recently demonstrated that the organism may survive the winter in sterilized and nonsterilized soil in Iowa. In this connection should be mentioned also the experiments of Kotila and Coons (9). They thoroughly inoculated nonsterilized soil in wooden pails in the fall and allowed part to overwinter in

a greenhouse and part out-of-doors. In the spring "instead of attempting the almost futile task of isolating the organism from the soil by the plating method, raw tuber slices were inoculated with small portions of the soil, thus allowing the tuber slices, the natural host, to do the selecting. One hundred and eight tuber slices were inoculated. . . . After five days, rotting was evident on a few slices and after a still longer incubation period, it was observed that ninety of the possible 108 tuber slices were rotting. It should be recorded that from the soils which were used as inoculum in this experiment, there were produced on the potato slices mixed cultures of bacteria and fungi, and that some of the slices showed discoloration not entirely dissimilar to the blackening which appears on tubers from blackleg. It is to be noted that a long time period elapsed before rotting began, many times greater than the regular period for blackleg bacteria to produce rot. Certain soil fungi, and associated bacteria, are capable of producing discoloration and rotting of tubers and these are likely to have been responsible for the rotting in this experiment. Attempts to isolate the blackleg organism directly from the rotting slices or from plants inoculated from the discolored tuber slices resulted in failure. It is doubtful,therefore, if any of the rotting was of the *B. atrosepticus* type. The conclusion, therefore, seems justified that under the conditions of this experiment, the blackleg organism did not overwinter in the soil, either in the greenhouse or out-of-doors."

In the opinion of the present writer, the above conclusion is not entirely justified. Kotila and Coons (9) show in this same paper that when tuber slices are inoculated with a very small quantity of bacteria no decay is evident before five days, while, when large quantities are used, the decay appeared in two days. Now it is to be expected that the blackleg pathogene, after overwintering in nonsterilized soil in competition with the natural soil flora, would be reduced to relatively small numbers. But, when such material is used as inoculum, they conclude, because the resulting decay is slow in appearing, that certain soil fungi and bacteria were responsible for the decay. In view of his own experiments, the writer is quite confident that had they continued their selective inoculations further they would have been able to reisolate the pathogene with little difficulty.

Both sterilized and nonsterilized soils have been used in the writer's overwintering experiments. Periodic isolations have shown that the relative abundance of the bateria decreases so rapidly in nonsterilized soils that after a few days it is difficult to reisolate the pathogene without resort to the selective method described above. In sterilized soils the decline is much slower and the bacteria are relatively abundant after many months of exposure.

Coons and Kotila (4) isolated a bacteriophage from the soil and concluded that in all probability it played a prominent part in the decline of

B. atrosepticus and *B. carotovorus* in the soil. The writer has not worked with this bacteriophage, but it is evident that whatever factor may be responsible for the decline, it does not result in the complete elimination of the blackleg pathogene.

Many workers have pointed out that the blackleg pathogene is resistant to low temperatures. Jennison (7) exposed the organism in sterilized soil for 24 hours to a temperature varying from −28° C. to −6.7° C. without any apparent injury. In the writer's experiment the bacteria have been exposed in sterile and nonsterile soils for an entire winter, during which the temperature was as low as −25° F. and remained below 0° F. for long periods. Although the bacteria apparently were reduced in abundance, some of them were not killed and they were readily reisolated.

Many investigators have called attention to the sensitiveness of the blackleg pathogene to desiccation. Morse (12), Smith (20), and others have shown that the organism is killed in a few hours if dried on the surface of glass at room temperature and humidity. Jennison (7), on the other hand, states that it is quite resistant to drying and remains long viable on plain beef agar. Kotila and Coons (9) found that, when dried on silk threads, the organism would survive four days, while, under the same conditions, it died within a few hours if dried on the surface of cover-glasses. They refer to the work of Giltner and Langworthy (5), who have shown that many non-spore-bearing bacteria are able to survive in air-dried soils for long periods, but Kotila and Coons did not report any experiments of this type. In so far as the writer could learn, no experiments have ever been reported in which the survival of the pathogene in dry soils has been tested. It was decided, therefore, to attempt to find out how long the pathogene would survive in soils stored in atmospheres of different degrees of relative humidity. Before this experiment was started a sample of soil was found in the laboratory which had been collected from a potato field near Crookston, Minnesota, and had been kept for more than a year in a loosely covered cardboard box. The soil was thoroughly dried and had a moisture content of 1.98 per cent. By using the selective method of isolation a vigorously pathogenic culture was isolated from this soil which in all salient characters was identical with authentic cultures of the blackleg pathogene. This alone wiuld indicate that the organism is able to survive at least a year in very dry soil. However, it was decided to make additional tests under controlled conditions.

Two types of soil were used, one the soil from Crookston, Minnesota, mentioned above, and the other a rich garden loam from a garden near London, England. The pH value of the former soil was found to be approximately 7.5 and of the latter 5.8. Each sample was oven-dried and then finely pulverized. Fourteen five-gram samples of each soil were prepared

and put into small test-tubes which were then plugged with cotton. Two tubes of each type of soil were placed in each of seven large-mouth bottles, the bottoms of which were padded with glass wool. The bottles were then plugged with cotton, placed in an autoclave, and sterilized at 20 pounds pressure for three hours on three successive days. Representative samples of the soil were then tested in broth and found sterile. Each tube was then inoculated by adding one cc. of a 24-hour broth culture of the blackleg pathogene.

Solutions of sulphuric acid and distilled water were prepared according to the table given by Stevens (22) for the production of relative humidities of 0, 9, 18.5, 38.0, 60.7, 80.5, and 100 per cent. One hundred cubic centimeters of these solutions were put into their respective bottles and the bottles were then corked, sealed with paraffin, and placed in a dark cupboard at room temperature. At intervals of approximately one month the soil in each of the tubes was tested for the presence of the pathogene by inoculating a tube of sterile broth with a loopful of soil. Two or more of the cultures obtained each time were tested for pathogenicity on potato tubers. The experiment was begun October 17, 1927, and was terminated June 15, 1928. On January 2, 1928, all the tubes were removed from the bottles and wrapped tightly in several thicknesses of wrapping paper and were kept in a handbag until January 12, when they were placed in bottles over fresh sulphuric-acid solutions. Unfortunately, no provision was made for determining the rate of desiccation, but the moisture contents of the soil samples were determined at the close of the experiment. It was evident from observation that the moisture content of some of the samples did not reach equilibrium until after December 21. Although the soils were very dry at the close of the experiment, it is possible that they would have become drier had the experiment been continued longer.

In testing the viability of the organism no counts were made to determine the numbers of bacteria present. It was obvious, however, that in the drier soils the bacteria were rapidly diminishing in number. This was indicated by the increased length of time required for the clouding of the broth and by the fact that on April 12 and June 15 it was necessary to use larger quantities of the drier soils before growth was obtained in the broth.

From the results of this experiment it is evident that the organism may survive in the soil under very dry conditions.

It remained viable in soils dried eight months over concentrated sulphuric acid. At the end of this period the moisture contents of the two soils were only 0.45 and 0.25 per cent. These results contradict the popular assumption that the blackleg pathogene is exceptionally sensitive to desiccation. They are, however, in accord with those of Giltner and Langworthy

(5) who found nonspore-forming bacteria surviving in soil moisture of less than one per cent. It seems extremely unlikely that any agricultural soil would in nature become so dry that the blackleg pathogene could not survive.

In comparison with the soil tests, a parallel series of tests were made with the silk-thread method described by Peltier (16) in his study of *Pseudomonas citri*. It was found that the bacteria were killed in less than 24 hours in all degrees of relative humidity under 100 per cent. Goss (6), in 1923, reported briefly that "Investigations on the viability of *B. phytophthorus* under various conditions of temperature and moisture have shown the organism to be very susceptible to desiccation. At 100 per cent humidity the organism remains viable for at least 8 days at all temperatures from 5° to 30° C. At 90 per cent humidity with a temperature of 25° C. it remained viable for only 3 hours. At 80 per cent humidity with the same temperatures the organism lived for only one hour." By personal correspondence it was learned that Goss used the silk-thread method to obtain these results. The writer's results obtained by this method agree with those reported by Goss, but it is evident that the results obtained with its use are not a reliable indicator of the resistance of the bacteria to desiccation in the soil.

It is difficult to say just what the factors are which enable the organism to survive in such extremely dry soil. Giltner and Langworthy think that the traces of hygroscopic moisture ever present about the soil particles are partly responsible, but they think there must be other factors. The water held by the soil colloids must certainly be of some importance. The very slow rate of drying in soils, which allows the bacteria time for becoming adapted to the dry conditions, is also a factor not to be overlooked.

The facts here presented force one to the conclusion that the blackleg pathogene can and does survive in the soil and is possibly very wide-spread in its occurrence, although no extensive survey has been made. When one considers this in connection with what has been demonstrated concerning the methods of infection in potato blackleg, it is realized that the incidence of the pathogene may not be so important in the development of the disease as are the proper conditions for inoculation and infection.

Kotila and Coons (9) have demonstrated by soil- and water-culture inoculations that "the usual method of blackleg infection is not through either injured or uninjured roots." The writer has repeated and verified these experiments. He has also examined hundreds of plants affected with blackleg in all stages of development, with the view of determining the avenue of infection; and, in more than ninety-nine per cent of the cases observed, the infection originated in the seed piece. As has been shown in a

previous paper (11), infection may be systemic in some cases, the seed tubers having been produced on diseased plants and infected through the decaying stolons. In other cases it can be proven that this type of infection is not involved. In some of these cases the seed-corn maggot has been shown by Leach (10) and Bonde (3) to play an important part as a common and effective agent of inoculation. It has been shown also that the insect can and frequently does disseminate the pathogene with its eggs. It appears from the facts presented here that this insect might also pick up the organism from the soil and successfully inoculate the seed pieces. On the other hand, although the pathogenic bacteria may be in the soil in great abundance, they usually are unable to infect the seed pieces without the aid of some such agent of inoculation, which enables them to penetrate the layer of wound cork formed by the seed piece.

If the conclusion that the blackleg pathogene survives in the soil is correct we would expect seed pieces planted under conditions inhibitory to cork formation to be more subject to the disease than those planted under more favorable conditions. There is abundant evidence to show that this is true. It is a matter of common observation that blackleg is most destructive in heavy, moist soils.

Morse (12) states that "more blackleg is observed in wet than in dry seasons" and that "all other things being equal, the disease is more likely to occur in wet than in dry soil, and is more prevalent when the early part of the growing season is characterized by abundant rainfall." He also describes one example in which the disease was first noted "near the center of the affected area from which it gradually spread outward. The season had been excessively wet, and this area coincided with a low, undrained pocket or depression in the field, where water would stand for a few hours after each heavy rainfall."

Murphy (13) says "more diseased plants are found in low-lying parts of a field than in higher." One example is given in which "It was found in extensive counts that there were three blackleg plants in the low-lying area to one in the higher."

The writer has made numerous observations of similar nature, and any one who has studied the disease to any extent will agree that outbreaks of the disease very frequently occur in water-logged soils. Where attempts have been made to explain this relationship, emphasis has been put on the growth requirements of the pathogene and very little attention has been paid to the influence of such conditions on the seed tubers. As pointed out above, it has been assumed generally that the pathogene is very susceptible to desiccation and that such moist soils were especially conducive to its growth. Without doubt such conditions are favorable to the growth of the pathogene

which is a facultative anaerobe, but, at the same time, one should not overlook the influence of water-logged soils on the formation of wound cork by the seed pieces. That abundant oxygen is necessary for the development of cork in the potato tuber has been shown by Kny (8), Appel (2), Olufsen (14), and others. It is obvious that excessive moisture in the soil inhibits cork formation, but apparently there are no records of a careful study of the phenomenon.

In order to obtain a more accurate measure of this influence, freshly cut Early Ohio tubers were planted in a series of jars or cylinders of quartz sand, each of which was held at a different moisture content. In the jars with the higher moisture contents, water was supplied from the bottom, and the differences in water content were obtained by varying the water level. In the drier jars, the water was supplied through Livingston atmometers buried in the center of the jar, sealed, and connected to a bottle of water by a rubber tube. The differences in moisture content were obtained by varying the height of the jar above the bottle of water. On the 1st, 2nd, 4th, 6th, and 8th days after planting, a tuber was removed from each jar and the cut surface was examined for the presence of wound cork. Sections were cut on a sliding microtome, stained in Sudan III, and mounted in glycerine for microscopic examination. In this way a very good idea of the effect of excessive soil moisture on the cork formation was obtained.

It is realized that the conditions of the experiment were not comparable in all respects to those in nature, for the influence of a given moisture content would vary with the type of soil. The results obtained show only the relative effect of different degrees of moisture and not the absolute effect of a given moisture content.

The experiment was made during the month of March when the tubers were no longer dormant. The results, as indicated by the amount of cork formed by the eighth day, are given in table 1.

The progress of healing as observed in the experiment agreed closely with the process described by previous investigators. During the first 48 hours the walls of the outer layer of cells became infiltrated with a dark brown substance which apparently makes them impervious to water. These cells do not readily stain with suberin stains. This process, sometimes termed "blocking," is considered a form of suberization and is inhibited by exclusion of oxygen. Beginning about the third day, under favorable conditions, the starch disappears from and cross walls are formed in the second or third layer of cells beneath the wounded surface. These walls all lie parallel to the wounded surface. It usually requires from 8 to 10 days for the formation and suberization of these new cells which make up the new periderm. It will be noted that in this experiment an excessive amount of

TABLE 1.—*The influence of the amount of moisture in the soil on the amount of wound cork formed by cut potato tubers in eight days*

Pot no.	Moisture content in per cent	Outer cells	New cross walls	
			No.	No. suberized
1	Less than 1	Suberized	2–4	2
2	2.39	do	5–8	3
3	5.91	do	5–8	3
4	7.88	do	5–8	3
5	9.38	do	4–6	2
6	12.03	Lightly suberized	1–4	None
7	12.59	do	1–4	do
8	14.75	Not suberized	None	Outer cells autolyzed

moisture resulted in a decided retardation or complete inhibition of these processes. In the more moist cylinders, not only was there no suberization or cell division, but the cells became soft and mushy, evidently because of asphyxiation and resulting autolysis.

Since the blackleg pathogene is a faculative anaerobe and may survive in the soil, it would seem that under such conditions infections could easily arise from the soil. It is very probable that such infection is largely responsible for the greater abundance of blackleg in excessively wet soils and in unusually rainy seasons. Of course, it is realized that the problem is not so simple as a consideration of these factors alone would imply. Certainly there are other factors which, acting under different conditions, would be of primary importance in determining the development of the disease. These are, however, not considered in the scope of this paper.

The marked influence of oxygen on wound-cork formation and on infection by the blackleg pathogene was further demonstrated by the following experiment. Several potato tubers were inoculated with a pure culture of the blackleg pathogene by the "hole and plug" method. The inoculated tubers were divided into three lots and placed in three separate moist chambers. The first container was a 3-gallon earthenware crock with a fairly loose-fitting top. The moisture was supplied by a layer of wet cotton on the bottom of the crock. The second was a large glass desiccator with a close-fitting top. The lower section was filled with water, and the inoculated tubers were suspended on a wire platform above the water. The third container was similar to the second but differed in that a stream of fresh air was drawn through the desiccator by means of two glass tubes sealed in the top of the vessel. The air-inlet tube extended to a point just below the tubers so that the air was drawn about the tubers and then removed from

the top. The air current was supplied by a suction pump and the air was bubbled through two flasks of water before it was drawn into the moist chamber. Saturation of the air in the chamber was insured by keeping the water in these flasks a few degrees warmer than the temperature in the chamber. In this way the warm, moist air was slightly cooled on entering the chamber and precipitated moisture was visible on the sides of the vessel throughout the duration of the experiment. The moist chambers were placed side by side on a table at room temperature.

In the first container the loose-fitting top permitted sufficient interchange of air to cause some fluctuation and variation in relative humidity, although it probably closely approached saturation at all times. The oxy-

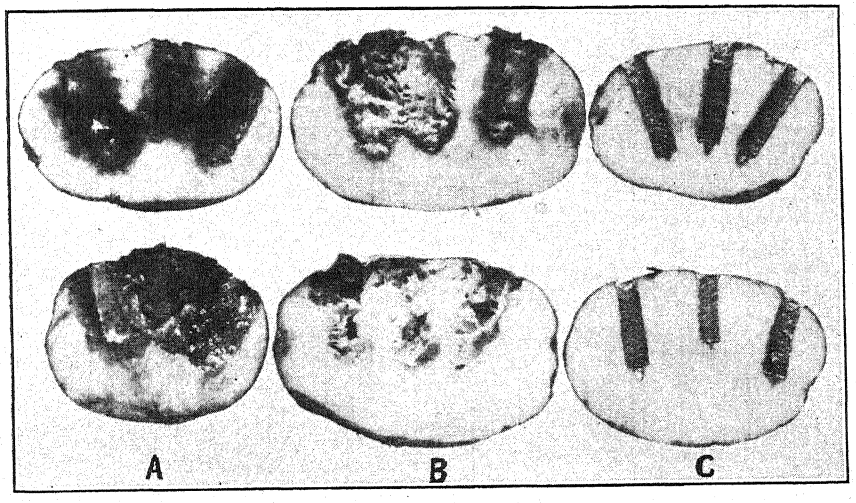


FIG. 1. The influence of oxygen supply on infection and decay of potato tubers by the blackleg pathogene. A. Tubers incubated in moist chamber with loose-fitting top. B. Tubers incubated in moist chamber with close-fitting top. C. Tubers incubated in a moist chamber in saturated atmosphere but with a constant supply of fresh air.

gen supply in the crock was soon consumed by the respiring tubers and was not entirely replaced by diffusion through the leak in the top.

In the second container the air was saturated with moisture at all times, but the oxygen consumed in respiration could not be replaced.

In the third container a saturated relative humidity combined with abundant oxygen supply prevailed.

After four days of incubation two tubers were removed from each container and cut in half to determine the amount of decay. These tubers are shown in figure 1. It will be seen that the tubers in the first two containers were badly decayed, while those in the container receiving a constant sup-

ply of fresh air had not decayed at all. The decayed tissue of the tubers incubated in the closed desiccator was almost pure white, showing only traces of browning along the margins. The influence of the limited supply of oxygen available in the crock was evident in the darker color of the decayed tissue of the tubers incubated in the crock.

Microtome sections were cut through the inoculated portions of the tubers shown in figure 1 and were examined for wound cork. Photographs of these sections are shown in figure 2. It was found that an abundance of

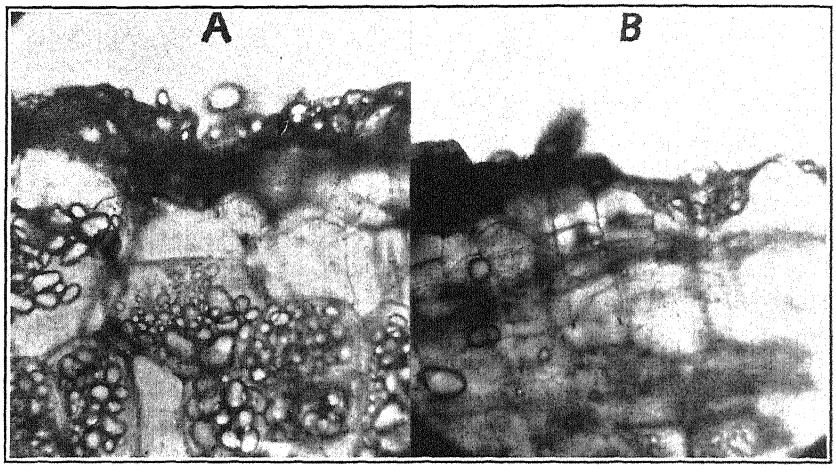


Fig. 2. The influence of oxygen supply on wound-cork formation by tuber inoculated with the blackleg pathogene and incubated in moist chambers. A. A photomicrograph of a section of the infection court of the tubers shown in figure 1, A. B. The same for figure 1, C.

wound cork had been formed by the tubers incubated in a constant supply of oxygen but that none had formed in the other tubers.

This experiment was repeated several times and it was found to be almost impossible to rot tubers with the blackleg pathogene when they were given a constant supply of fresh air, even when incubated at the optimum temperature and in a saturated atmosphere.

The influence of oxygen on infection and development of blackleg decay, as brought out in this experiment, should be of some practical importance in the storage of potatoes. It is limited, however, in its application by the fact that the constant replacement of oxygen stimulates respiration and the resulting sprouting of the tubers.

An appreciation of this relationship is also of significance in experimental work. It has been observed, for example, that when a single tuber is inoculated and placed alone in a large moist chamber it will frequently

escape infection. On the other hand, if the moist chamber is filled with tubers so that the oxygen supply is soon exhausted by respiration, practically 100 per cent infection is obtained. A failure to recognize this influence of the oxygen supply may have been responsible for many of the variable results reported in literature dealing with bacterial decay of potato tubers.

SUMMARY

1. The pathogene causing potato blackleg can and commonly does survive the winter in the soil in Minnesota.

2. The conclusion of earlier workers that this organism could not survive the winter in the soil is not justified. Their conclusion was based on negative evidence which loses its force in the light of recent investigations on the methods of infection.

3. The pathogene is resistant to the very low temperatures of a Minnesota winter.

4. Experiments in which the organism was dried slowly in soils proved it to be extremely resistant to desiccation. The conditions of these experiments are considered to be more comparable to those occurring in nature than those in which the bacteria are dried on cover glasses or silk threads.

5. Excessive soil moisture greatly inhibits the formation of cork. This inhibition is probably due to the exclusion of oxygen. The blackleg pathogene, being a facultative anaerobe, grows readily under such conditions. It is concluded that under such conditions blackleg may arise by infection from the soil. It is also concluded that the inhibition of cork formation by the exclusion of oxygen is largely responsible for the more abundant development of blackleg in excessively wet soils or in very rainy seasons.

6. All the evidence obtained by a study of potato blackleg over a series of years leads to the conclusion that the presence or absence of the proper conditions for infection, or of some agent of inoculation, may be of greater importance as a limiting factor in the development of the disease than the incidence of the pathogene.

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PHYSIOLOGIC FORMS OF BARLEY MILDEW, *ERYSIPHE GRAMINIS HORDEI* MARCHAL

E. B. MAINS AND S. M. DIETZ[1]

Physiologic specialization of rusts of grasses has long been known. However, it was not until 1902 that Marchal (2) first pointed out the existence of physiologic specialization in the powdery mildew (*Erysiphe graminis* DC.) of grasses.

He distinguished seven races of *Erysiphe graminis* based on their host specialization: *Tritici* on species of Triticum, *Hordei* on species of Hordeum, *Secalis* on species of Secale, *Avenae* on species of Avena, *Poae* on species of Poa, *Agropyri* on species of Agropyron, and *Bromi* on species of Bromus. He found that conidia of the mildew from *Hordeum vulgare* would infect *Hordeum vulgare, H. hexastichon, H. trifurcatum, H. distichon, H. Zeocriton, H. nudum, H. jubatum, H. murinum* but not *Agropyron repens, A. caninum, A. giganteum; Agrostis alba, Alopecurus pratensis, Andropogon Ischaemon, Anthoxanthum odoratum, Aira caryophyllea, Avena sativa, A. orientalis, A. fatua; Arrhenatherum elatius Brachypodium sylvaticum, Briza media, Bromus sterilis, B. patulus, B. mollis, B. racemosus, B. secalinus, B. arduennensis, B. squarrosus, B. macrostachys, Cynosurus cristatus, Dactylis glomerata, Deschampsia flexuosa, Elymus arenarius, Festuca rubra, F. elatior, F. gigantea; Holcus lanatus, Koehleria cristata, Lolium perenne, Melica ciliata, Milium effusum, Phleum pratense, P. Boehmeri; Poa annua, P. nemoralis, P. serotina, P. pratensis, P. mulalensis, P. trivalis; Secale cereale, Setaria viridis, Trisetum flavescens, Triticum vulgare, T. Spelta, T. polonicum.* Similar results (3) were obtained when ascospores were used.

Both Reed and Salmon have studied the race *Hordei.* Salmon (5) was able to infect *H. decepiens, H. distichon, H. hexastichon, H. intermedium, H. vulgare,* and *H. Zeocriton.* A slight infection was obtained on *H. bulbosum* and *H. maritimum.* He was not able to infect *H. jubatum, H. murinum, H. secalinum, H. silvaticum, Avena sativa, Triticum vulgare,* nor *Secale cereale.* Reed (4) infected *H. distichon* L., *H. nudum* L., *H. steudelii* x *trifurcatum, H. tetrastichon* L., *H. trifurcatum* Jacq., *H. vulgare* L., *H. Zeocriton* L., and young seedlings of *H. nodosum* L. Negative results were obtained with *H. bulbosum* L., *H. maritimum* With., *H. jubatum* L., *Triticum vulgare* Vill., *T. dicoccum* Schrank, *Triticum durum*

[1] Published with the approval of the Directors as a joint contribution from the Department of Botany of the Purdue University Agricultural Experiment Station, LaFayette, Ind., and the Section of Botany and Plant Pathology, Iowa Agricultural Experiment Station, Ames, Iowa.

Desf., *Avena sativa* L., and *Secale cereale* L. From the studies of Marchal, Salmon, and Reed, therefore, it would appear that the race *Hordei* is rather closely restricted to a few closely related species of Hordeum.

Salmon and Reed made their studies with the powdery mildew from various species of Hordeum. They found little evidence of differences in pathogenicity of the mildew from such different sources. Their results, therefore, would indicate that the *Hordei* race was rather uniform in its pathogenicity. They, however, apparently did not study *Erysiphe graminis hordei* from a number of different localities. Apparently, their studies were for the most part confined to relatively few varieties of barley.

Salmon, however, devoted some attention to the question of whether any of the species or varieties of barley showed differences in susceptibility to mildew. In studies conducted in the greenhouse where plants were inoculated under bell-jars only the botanical varieties *Hordeum vulgare* var. *leiorrhynchum* and *H. vulgare* var. *himalayense* showed any signs of resistance. He found from a study under field conditions in 1903 when powdery mildew was abundant at Cambridge, England, that *Hordeum vulgare* var. *himalayense, Walpersii, crispum; H. Zeocriton* var. *melanzeocriton; H. distichon* var. *pictum, eingens, medicum, persicum, laxum, ianthinium, ramulosum, nigrosubinerme, Rimpaui, H. decepiens* var. *triceros, nudideficiens; H. hexastichon* var. *hexasticho-ramosum; H. spontaneum* showed no mildew. Some varieties showed only a trace, while others developed a fair amount and still others were excessively mildewed.

MATERIALS AND METHODS

The studies described in this paper are an outgrowth of extensive studies of varieties of barley for reaction to leaf rust, *Puccinia anomala*, which are cooperative investigations between the Office of Cereal Crops and Diseases of the United States Department of Agriculture and the Botanical Department of the Purdue University Agricultural Experiment Station. During the winter of 1923–1924, 655 selections and varieties received from the Office of Cereal Crops and Diseases were studied in the greenhouse of the Agricultural Experiment Station of Purdue University for reaction to leaf rust. Considerable mildew developed on these varieties during the study for rust reaction and it was noted that apparently marked differences occurred in the reaction of varieties to mildew. Therefore it was decided to inoculate all the varieties with mildew after notes concerning rust had been obtained. As a result complete notes were obtained concerning both rust and mildew and marked differences in the reactions were found to exist.

In 1925, 1926, 1927, and 1928 these studies were continued on a selected series of varieties at Purdue University. In 1926 a selected set of

varieties was furnished the Section of Botany and Plant Pathology of the Iowa Agricultural Experiment Station for similar studies of powdery mildew where studies were made in 1926, 1927, and 1928.[2]

It is not proposed in this paper to attempt to give all of the data accumulated during the five years. One of the results of this study has been the discovery of the existence of physiologic forms, which may be distinguished by the use of a few of the varieties studied. These, together with a few others, outstanding for their general resistance or susceptibility to the various cultures of mildew, have been selected for discussion.

These varieties were grown in four-inch pots in the greenhouse during the winter months and were inoculated in the second- to fourth-leaf stage of development. The cultures of mildew were carried on some susceptible variety, such as Oderbrucker, C. I. 940.

The varieties were inoculated by shaking potted plants of heavily-mildewed-susceptible varieties over pots of seedlings of the varieties to be tested, thus dusting such seedlings with conidia. The seedlings were then atomized with a fine spray of water and covered with wet muslin-cloth or placed in a moist chamber for 24 hours. Notes were taken when the development of the mildew had reached its maximum, usually in 7 to 14 days after inoculation.

Five classes of reaction were distinguished, as in the case of the powdery mildew of rye (1). These classes are as follows:

Highly resistant, denoted as 0. Macroscopically, no mycelium is evident. Chlorotic or necrotic spots may be developed by some varieties. Microscopically, a slight amount of mycelium may develop and infection may be evident macroscopically by faint flecks. (Fig. 1, A–C.)

Very resistant, denoted as 1. Slight to moderate development of mycelium evident macroscopically but with little or no sporulation. (Fig. 1, D and E.) Chlorotic or necrotic spots developed by some varieties.

Moderately resistant, denoted by 2. A moderate to abundant development of mycelium occurs, accompanied by a slight production of conidia, figure 1, F and G. Chlorotic or necrotic areas are formed by some varieties.

Moderately susceptible, denoted by 3. A moderate to abundant development of mycelium occurs, accompanied by moderate sporulation. Figure 1, H.

Very susceptible, denoted by 4. Abundant mycelium is developed, accompanied by abundant sporulation. Figure 1, I and J.

[2] The writers are indebted to Dr. H. V. Harlan, who brought this extensive collection of varieties together from various parts of the world. We also wish to express our thanks to Dr. M. N. Pope, who kindly furnished us with the seed, and to Miss Mary L. Martini, who has furnished information concerning the classification of the varieties, and, further, to Mr. Leroy Compton, Mr. L. D. Leach, and Mr. H. C. Murphy, who assisted in the inoculations at Purdue University and Iowa State College.

RESULTS

During the winter of 1923–1924, the culture of mildew occurring in the greenhouse of the Department of Botany, Purdue Agricultural Experiment Station at LaFayette, Indiana, was studied on a large series of varieties. During the winter of 1924–1925, mildew in the same greenhouses was again studied on a selected set of varieties. The results obtained agreed very closely with those of the previous year. Twice during the winter of 1925–1926 and again in 1926–1927 the selected varieties were tested to mildew carried over from the previous year and results very similar to those of the previous years were obtained. These are summarized in table 1 under physiologic form 1. The varieties Arlington 702, Goldfoil 928, Hanna 906, C. I. 2444, and C. I. 1080 were outstanding for their marked resistance, being consistently of type 0. The varieties Kwan 1016 and Duplex 2433 showed a slight development of mycelium but no sporulation and are classed as type 1–. Consul 1061, Oswong 697, and C. I. 2416 showed still more development of mildew, being, however, very resistant, type 1. Some of the varieties showed more variation in reaction than others. Such varieties as Chilean D, Peruvian 1131, Turkestan 711, Bolivia 1257, Coast 276, Common Chile 663, Chilean C 1432, Abyssinian 1243, Luth 972, Peruvian 935, Lynch 919, and C. I. 1021 varied in reaction from type 0 to 1. Abyssinian 362, Hanna 966, Purple Nepal 1373, Black Hullless 1032, Nepal 595, and Heil's Hanna 682 varied from 1 to 2. Palestine 939, Blackhull 878, Arequipa 1256, Peru 653, Lehor 866, and Black Hullless 666 showed more variation, sometimes giving a reaction of type 0 and, at other times, type 1 or 2. Juliaca 1114 varied from type 2–3 and Nepal 475 from type 1–3. Oderbrucker 940 and Hooded Spring 716, while susceptible, showed some variation from type 3–4. Odessa 182 and Bohemia 204 represent a large number of highly-susceptible varieties, showing a consistent reaction of type 4.

This culture of mildew was lost during the summer of 1927 and no barley mildew occurred in the greenhouses at LaFayette, Indiana, during the winter of 1927–1928. In the autumn of 1928 powdery mildew was found on some plants of volunteer barley at LaFayette, Indiana, and was again studied upon the series of varieties used previous years. To this culture, several of the varieties showed considerably more susceptibility than to the cultures of previous years. The results of three tests during the winter of 1928–1929 are given in table 1 under physiologic form 2. Most of the varieties gave a reaction to form 2 very similar to that to form 1. However, Oswong 697, Purple Nepal 1373, Black Hullless 1032, Nepal 595, Nepal 475, and Heil's Hanna 682 showed considerably more susceptibility than to physiologic form 1, as can be seen by consulting table 1.

TABLE 1.—*Reaction of forty varieties of barley to five physiologic forms of powdery mildew, Erysiphe graminis hordei*

Variety[a]	C. I.[b] No.	Type of reaction to				
		P. f. 1	P. f. 2	P. f. 3	P. f. 4	P. f. 5
Hordeum vulgare pallidum						
Duplex	2433	1–	0	0	0	0
Kwan	1016	1–	0	0–1	0–1	1–2
Chilean D	1433	0–1	1	1	2	0–1
	1021	0–1	0	0	2	2
Coast	276	0–1	1–2	1	2	1
Chilean C	1432	0–1	1+	1+	2	1
Abyssinian	1243	0–1	2	1+	2	1
Common Chile	663	0–1	1+	2+	1–2	0–1
Peruvian	1131	0–1	0–1	2–3	1	3
Turkestan	711	0–1	0–1	2–3	1	2–3+
Bolivia	1257	0–1	1	1+	2–3	2–4
Luth	972	0–1	0–1	2–3	1	2–3
Consul	1061	1	1	0	3	1–2
Peruvian	935	0–1	1	3–4	1	4
Lynch	919	0–1	0–1	3–4	1	3–4
Arequipa	1256	0–2	1	3–4	2	4
Peru	653	0–2	1–2	1+	3	3–4
Juliaca	1114	2–3	2+	1+	4	4
Oderbrucker	940	3–4	4	4	4	4
Odessa	182	4	4	4	4	4
Hordeum vulgare nigrum						
Oswong	697	1	3	3–4	2	3
Hordeum vulgare hors-fordianum						
Hooded Spring	716	3–4	3–4	4	4	4
Hordeum vulgare coeleste						
Black Hullless	666	0–2	1–2	3+	4	3
Hordeum vulgare trifurcatum						
Lehor	866	0–2	2	2–3	4	3–4
Purple Nepal	1373	1–2	3+	4	4	3–4
Black Hullless	1032	1–2	3+	3+	4	4
Nepal	595	1–2	4	4	4	4
Nepal	475	1–3	4	4	4	4
Hordeum intermedium haxtoni						
Arlington	702	0	0	0	1	0
	1080	0	1+	0	3	1–2
Hordeum intermedium mortoni	2444	0	0	0–1	0–1	1
Abyssinian intermediate	2416	1	1+	1+	2	3–4
Hordeum distichon palmella						
Palestine	939	0–2	1	1	1	1
Goldfoil	928	0	0	0	0	4
Hanna	906	0	0	0	0	4
Hanna	966	1–2	1	3	0	3–4
Heil's Hanna 3	682	1–2	4	4	3–4	4
Bohemia	204	4	4	4	4	4
Hordeum deficiens steudeli						
Abyssinian	362	1–2	1+	1	2	1
Blackhull	878	0–2	1	3–4	1	4

[a] Varieties classified according to H. V. Harlan. The Identification of Varieties of Barley, U. S. Dept. Agr. Bul. 622. 1918.

[b] Accession number of the Office of Cereal Crops and Diseases, United States Department of Agriculture.

In the autumn of 1928 a collection of barley mildew was received from Dr. J. G. Dickson from Madison, Wisconsin. This was carried in a greenhouse compartment separate from the preceding and sown upon a similar set of varieties. Three different tests were made and the results showed this to be a physiologic form distinct from forms 1 and 2. The results of these studies are given under physiologic form 3 of table 1. As can be noted, this form is sharply separated from physiologic forms 1 and 2 by the reaction of Peruvian 935 and Lynch 919 (Fig. 2, A3 and B3), which showed a reaction of type 3 or 4 as contrasted with the types 0–1 shown to physiologic forms 1 and 2. The varieties Hanna 966 (Fig. 2, A4 and B4), Blackhull 878, Arequipa 1256 (Fig. 1, I), and Black Hullless 666 showed considerably more susceptibility to physiologic form 3 than to either of the other forms, and the varieties Oswong 697, Purple Nepal 1373, Black Hullless 1032, Nepal 595, Nepal 475, and Heil's Hanna 682 (Fig. 1, J) were considerably more susceptible than to physiologic form 1, but approached physiologic form 2 in their reactions.

In 1926 a collection of powdery mildew was made at Moscow, Idaho, and studied in the greenhouses of the Section of Botany and Plant Pathology of the Iowa Agricultural Experiment Station on the varieties used in the studies at Purdue University. This culture proved to be distinct from the three forms previously studied. In 1927 a collection was made in California, which proved to be very similar to the collection from Idaho. The results obtained from these studies are given in table 1 under physiologic form 4. The varieties Juliaca 1114, Lehor 866, Peru 653, Consul 1061, and C. I. 1080 were more susceptible to this form than to physiologic forms 1, 2 and 3. The reaction of Peruvian 935, Lynch 919, Blackhull 878, and Arequipa 1257 to form 4 resembled their reaction to physiologic forms 1 and 2, and they were more resistant to form 4 than to form 3. Hanna 966 showed considerably more resistance to form 4 than to forms 1, 2, and 3. Heil's Hanna 682, on the other hand, was more or less susceptible, types 3–4, to form 4, thus showing a reaction similar to its reaction to forms 2 and 3 but differing from its reaction to form 1 to which it was resistant, types 1–2.

In the summer of 1928 a culture of barley mildew was obtained at Ames, Iowa, which gave markedly different reactions in studies made during the winter of 1928–1929 at Iowa State College. The results of this study are given in table 1 under the heading physiologic form 5. This physiologic form is easily separated from the other four forms by the reactions of Goldfoil 928 and Hanna 906 (Fig. 3), which were very susceptible, type 4, to physiologic form 5 and highly resistant, type 0, to all the other forms. The unnamed variety C. I. 2416 was also much more susceptible to form 5 than to the other forms. Peruvian 935, Lynch 919, Blackhull 878, and Arequipa

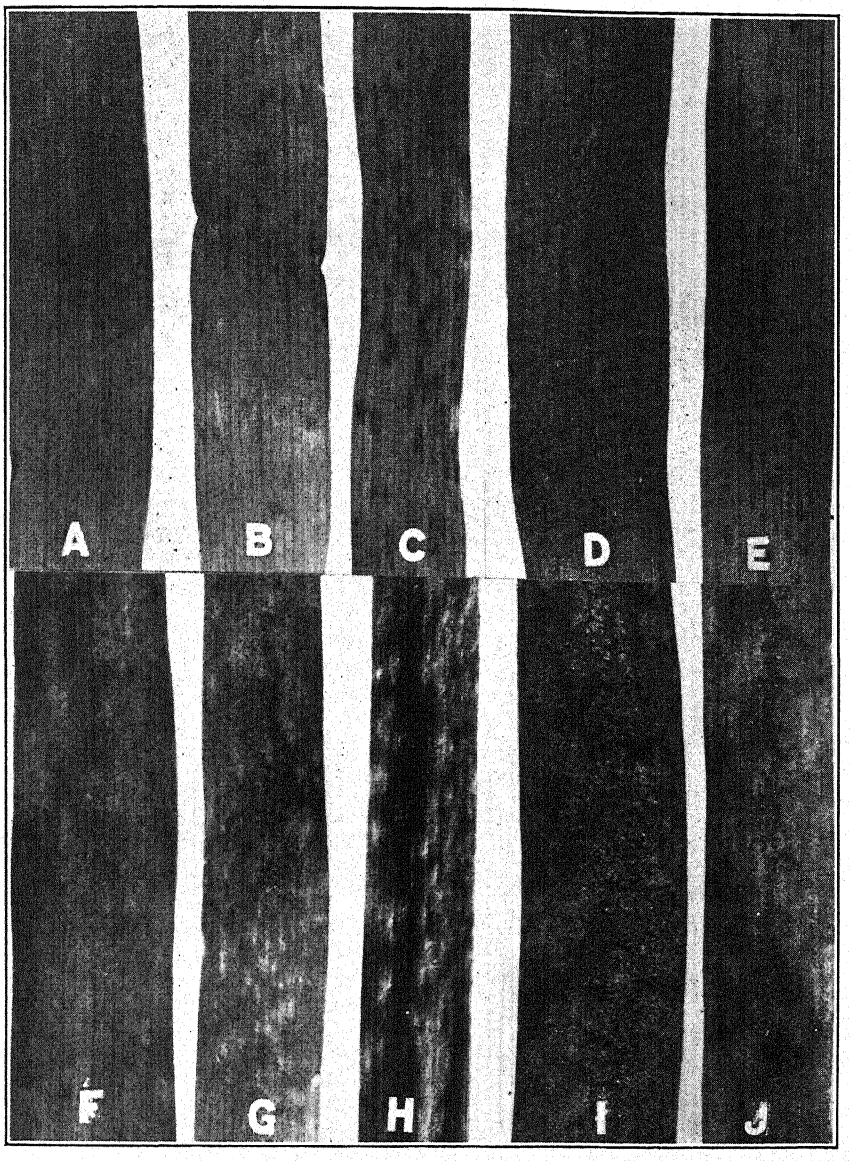


FIG. 1. Types of reaction of barley varieties to *Erysiphe graminis hordei* p. f. 3. A and B. Type 0, highly resistant, slight flecking. C. Type 0, highly resistant with definite necrotic spots. D and E. Type 1, very resistant, slight development of mycelium, and very slight sporulation. F and G. Type 2, moderate development of mycelium and slight sporulation. H. Type 3, moderately susceptible, moderate development of mycelium, and moderate sporulation. I and J. Very susceptible, abundant development of mycelium and sporulation. A. Arlington C. I. 702; B. Consul C. I. 1061; C. C. I. 1080; D. Abyssinian C. I. 362; E. Palestine C. I. 939; F. Luth C. I. 972; G. Peruvian C. I. 1131; H. Hanna C. I. 966; I. Arequipa C. I. 1256; J. Heil's Hanna No. 3, C. I. 682.

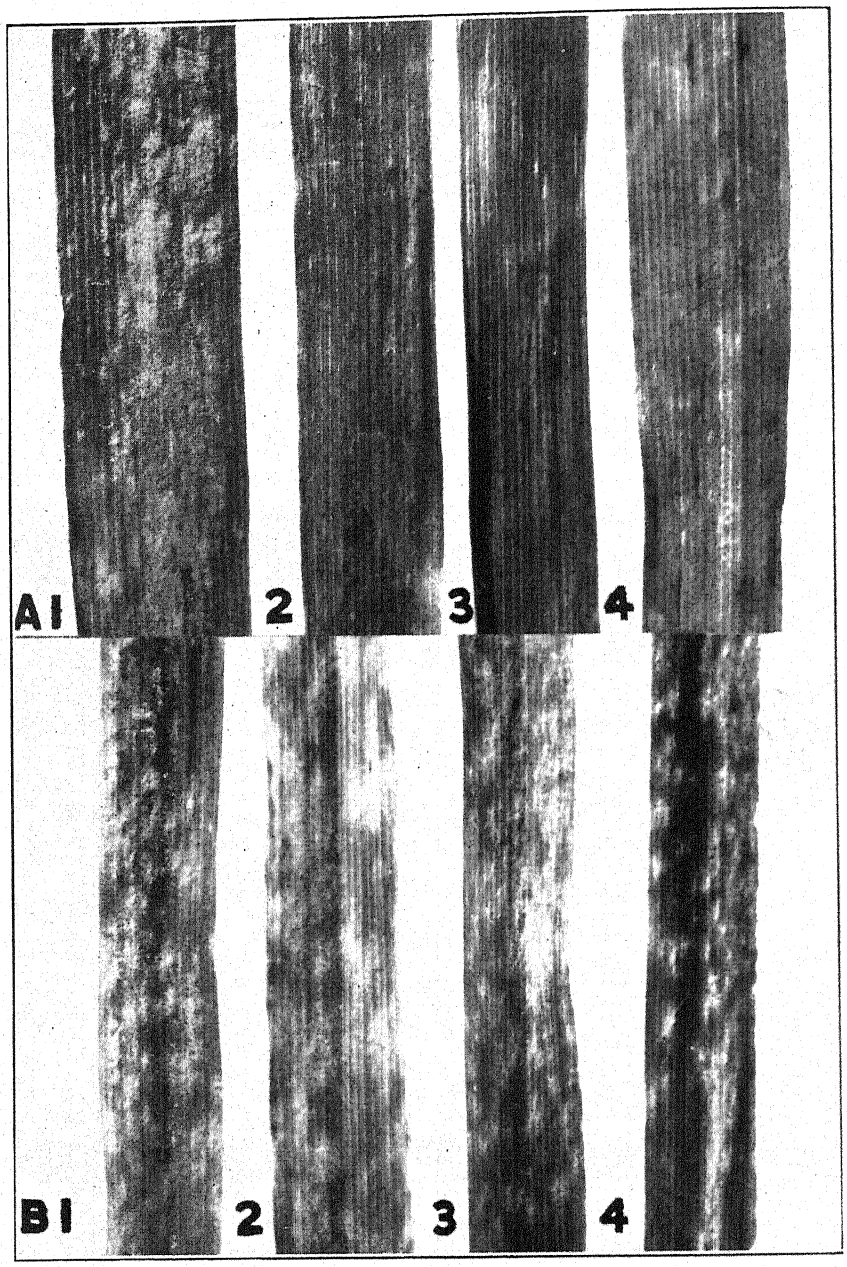


FIG. 2. Reaction of four varieties of barley to two physiologic forms of powdery mildew. A. Four varieties inoculated with physiologic form 2. B. Same four varieties inoculated with physiologic form 3. (1) Oderbrucker C. I. 940, susceptible to both forms. (2) Turkestan C. I. 711, highly resistant to p. f. 2 and moderately resistant to p. f. 3. (3) Lynch C. I. 919, highly resistant to p. f. 2 and susceptible to p. f. 3. (4) Hanna C. I. 966, very resistant to p. f. 2 and moderately susceptible to p. f. 3.

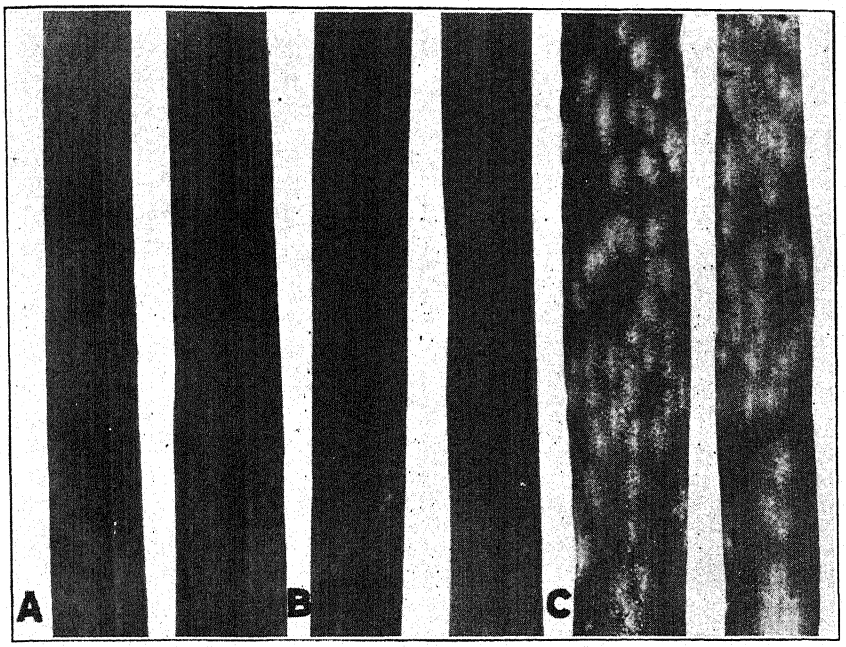


FIG. 3. Reaction of Hanna C. I. 906 to three physiologic forms of powdery mildew. A. Two leaves of Hanna inoculated with p. f. 2, highly resistant. B. Two leaves inoculated with p. f. 3, highly resistant. C. Two leaves inoculated with p. f. 5, very susceptible.

1256 were susceptible to form 5, thus giving a reaction similar to that to form 3 and differing from that to forms 1, 2, and 4 to which these varieties were resistant. Consul 1061 and C. I. 1080 were more resistant than to form 4 and somewhat less resistant than to forms 1, 2, and 3. Peru 653, Juliaca 1114, and Lehor 866, which were more or less resistant to forms 1, 2, and 3, were susceptible to both 4 and 5. Black Hullless 666 was more or less susceptible to forms 3, 4, and 5 and resistant to forms 1 and 2. Purple Nepal 1373, Black Hullless 1032, Nepal 595, Nepal 475, and Heil's Hanna 682 were resistant only to form 1 and susceptible to the other forms.

The five physiologic forms can be most easily separated by using four barley varieties as follows:

Nepal C. I. 595 resistant (1–2) — p. f. 1
Nepal C. I. 595 susceptible (4)
  Peruvian C. I. 935 susceptible (3–4)
    Goldfoil C. I. 928 susceptible (4) — p. f. 5
    Goldfoil C. I. 928 resistant (0) — p. f. 3
  Peruvian C. I. 935 resistant (0–1)

Black Hullless C. I. 666 resistant (1–2) p. f. 2
Black Hullless C. I. 666 susceptible (4) p. f. 4

As shown in table 1, the varieties Arlington 702, Duplex 2433, and C. I. 2444 are outstanding for their marked resistance to all five of the physiologic forms. These varieties are closely followed in resistance by Kwan 1016, Chilean D 433, C. I. 1021, Peruvian 1131, Turkestan 711, Bolivia 1257, Palestine 939, Abyssinian 362, Coast 276, Common Chile 663, Chilean C 1432, Abyssinian 1243, and Luth 972, varieties more or less resistant to all five of the physiologic forms.

The varieties Goldfoil 928 and Hanna 906 are very resistant to four physiologic forms and susceptible to one. Consul 1061 and the unnamed varieties C. I. 1080 and C. I. 2416 are more or less resistant to four physiologic forms and susceptible to one. Peruvian 935, Lynch 919, Blackhull 878, Arequipa 1256, Hanna 966, Peru 653, Juliaca 1114, and Lehor 866 are more or less resistant to three physiologic forms and more or less susceptible to two. Black Hullless 666 and Oswong 697 are more or less resistant to two physiologic forms and susceptible to 3. Purple Nepal 1373, Black Hullless 1032, Nepal 595, Nepal 475, and Heil's Hanna 682 are resistant to only one physiologic form and more or less susceptible to the other four. Oderbrucker 940, Odessa 182, Bohemia 204, and Hooded Spring 716 are included as examples of a large number of varieties that are susceptible to all of the physiologic forms.

The varieties resistant to all five of the physiologic forms are not restricted to any one species or group. They are distributed in all the major species, *Hordeum vulgare, Hordeum intermedium, Hordeum distichon,* and *Hordeum deficiens,* table 1. A similar situation also occurs even in agronomic varieties, as is shown by the reactions of the various selections of Hanna to physiologic form 3, Hanna 906 registering a type 0 reaction, Hanna 966 giving a reaction of type 3, and Heil's Hanna 682 giving a reaction of type 4.

DISCUSSION

These studies show that the various species of barley, *Hordeum vulgare, H. intermedium, H. distichon,* and *H. deficiens* are not uniformly favorable hosts for *Erysiphe graminis hordei.* While a large number of susceptible varieties occur in all of these species, a number of resistant varieties also are found in each. These resistant varieties also differ considerably. Some show no macroscopic signs of infection; some allow a slight development of mycelium with little or no sporulation; while others develop a slight to moderate sporulation.

Not only is it evident that the species *Erysiphe graminis* can be separated into races by the species of hosts which such races are able to infect,

but that the race *Hordei* can be still further subdivided into physiologic forms. Such physiologic forms can be sharply separated by the reaction of certain selections of barley. On the parasitic relationship, therefore, both the hosts and the parasites can be readily separated into groups by their reactions with each other. A situation, therefore, exists in this group similar to that which occurs in several other groups of parasitic fungi, such as the rusts and smuts.

It is evident that the existence of physiologic forms complicates exceedingly the problem of controlling the powdery mildew of barley with resistant varieties. Before this can be done with any degree of certainty it will be desirable to have the fullest possible knowledge of the extant physiologic forms and of the relative susceptibility of barley varieties not only in the seedling stage but under different environmental conditions and stages of development of the barley plant.

SUMMARY

1. The race of powdery mildew, *Erysiphe graminis hordei,* on barley contains at least five physiologic forms.

2. These physiologic forms can be separated by the reaction of a selected set of barley varieties resistant to some of the physiologic forms and susceptible to the others in the seedling stage in the greenhouse.

3. Barley varieties resistant to these physiologic forms have been found in all of the principal species, *Hordeum vulgare, H. intermedium, H. distichon,* and *H. deficiens.*

4. Of the 40 varieties discussed in this paper, three have been found very resistant to all five of the physiologic forms. Sixteen showed more or less resistance to all five physiologic forms. Five varieties were resistant to four forms and susceptible to one. Eight varieties were resistant to three forms and susceptible to two. Two varieties were resistant to two forms and susceptible to three. Five varieties were resistant to one form and susceptible to four, while four varieties were susceptible to all five physiologic forms.

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CULTURAL CHARACTERISTICS OF PHYSIOLOGIC FORMS OF SPHACELOTHECA SORGHI[1]

C. H. Ficke and C. O. Johnston

INTRODUCTION

The covered kernel smut of sorghum (*Sphacelotheca sorghi* (Link) Clinton) is the most destructive disease of grain sorghums in the southern Great Plains area. Prior to 1923 varieties of milo and feterita seemed to be free from this disease and it was not considered necessary to treat the seed for smut. In 1923 and 1924 specimens of kernel smut were collected in fields of milo in Kansas, New Mexico, and Texas. In 1921 a small percentage of smut was found in feterita at Chillicothe, Texas. Tisdale, Melchers, and Clemmer (11) studied the pathogenicity of three strains of the covered kernel smut, which they designated as the common, milo, and feterita strains. They reported that the common strain attacked many varieties of sorghum, but failed to infect milo and feterita. The milo form infected milo and several other varieties, but failed to infect feterita. The feterita strain infected feterita, but failed to infect milo. Thus they proved that the three strains were distinct and probably were physiologic forms of the fungus causing covered kernel smut.

The studies herein reported were conducted in the laboratory of the Kansas Agricultural Experiment Station during the winter of 1928–29. The results of these experiments show that the three strains mentioned above also can be distinguished by cultural characteristics on various kinds of nutrient media and indicate that covered kernel smut is caused by at least three definite physiologic forms of *S. sorghi*. For convenience these forms have been assigned numbers and reference to them hereafter will be by number. The common covered kernel smut, the milo, and the feterita forms discussed by Tisdale, Melchers, and Clemmer will be referred to as physiologic forms 1, 2, and 3, respectively.

Christensen and Stakman (2) described fifteen physiologic forms of *Ustilago zeae* (Beckm.) Ung. that were distinguishable by such cultural characters as rate of growth, color, topography, surface, zonation, conidial production, and margin. At least seven of these could be recognized by their parasitic behavior on corn. More recently Rodenhiser (8) reported that he was able to distinguish fourteen physiologic forms of *Ustilago tritici* (Pers.) Jens., twelve of *U. nuda* (Jens.) K. and S., seven of *U. hordei*

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(Pers.) K. and S., five of *U. levis* (K. & S.) Mag., and eighteen of *U. avenae* (Pers.) Jens., in culture by color, topography, surface, consistency, and type of margin. These could be distinguished only on the proper media and under proper environmental conditions.

MATERIALS AND METHODS

Smutted heads from typical plants of Dwarf Yellow milo, feterita, and Pink kafir were selected as sources of smut. Previous experiments in the field had proved that the three strains of smut selected for these studies were pathologically distinct and probably were distinct physiologic forms. The varieties of sorghum on which these forms developed had been inbred for several generations and were known to be pure for varietal characters and reaction to covered kernel smut. As soon as the smutted heads emerged from the boot they were bagged, tagged, and then kept covered until the smut was well developed. At maturity the heads were cut from the stalks without removing the bags and dried. Those infected with each strain of smut were stored separately. There was thus virtually no opportunity for mixture or contamination in the field or in harvesting and storage.

When needed for culturing the heads were removed from the bags one at a time and unruptured smut galls selected on each. There seemed to be no consistent differences in shape, size, or color of the sori produced by the three strains. The enclosing membrane of the smut gall was disinfected and a portion of it carefully removed. A small quantity of the chlamydospores was taken from the interior with a sterile needle. These spores were immediately transferred to test-tubes of liquid carrot agar from which poured plates were made. Some multichlamydospore cultures were also obtained by making direct transfers of spore material from the smut gall to carrot-agar slants. Previous cultural experiments with *U. zeae* and other smuts had shown this medium to be very satisfactory for the culturing of smut fungi. Several multispore cultures were made from each form of the smut in this manner and kept in the laboratory at room temperature.

After a few days' growth there was evidence that the strains under investigation were culturally distinct. Subcultures immediately were made by transferring to fresh plates. At the same time a new series of cultures was made from the smutted heads. These latter proved to have the same general characteristics as the first series of cultures.

It seemed desirable to ascertain whether differences between the forms could be distinguished on media other than carrot agar. Transfers, accordingly, were made to poured plates of potato-dextrose, oatmeal, and carrot agars. As will be pointed out later, distinct differences could be distinguished between all of the forms on the three kinds of agar. All of these operations were repeated several times to make sure that the dif-

ferences were not merely chance variations. With but one exception in form 2, multispore cultures of forms 2 and 3 remained constant for their general cultural characteristics on each of the media. They had somewhat different characters on the various media, however. In short, they seemed to be very stable and to give the same reactions repeatedly. Form 1, on the other hand, seemed relatively unstable and numerous wedge-shape or fan-shape sectors developed in it, especially on carrot agar. These sectors often had characters strikingly different from those of the parent culture.

The origin of the sectors in cultures might be explained on the basis of a mixture of forms, mutations, or heterothallism. Several series of cultures from single sporidia and single chlamydospores, respectively, were made from each of the forms of smut studied. Transfers of each of the forms were made from stock cultures on agar slants to Erlenmeyer flasks of carrot decoction. The cultures thus established were allowed to develop until the medium became cloudy with conidia. Very sparse sowings of conidia then were made in poured plates of carrot agar by the dilution method. Single isolated conidia were located, marked, and transferred to fresh plates as soon as cultures from these were macroscopically visible. Many single conidium cultures were thus established. It has been mentioned that the multichlamydospore cultures were obtained by making direct transfers of spore material from the smut gall to carrot-agar slants. The single chlamydospore cultures were obtained by the dilution plate method. All cultures were kept at room temperature, about 80° F. The single chlamydospore cultures as well as the multispore cultures were grown on carrot agar, potato-dextrose agar, and oatmeal agar. The single conidial cultures, with the exception of those of form 1, were grown on carrot agar only. The cultural characteristics of the three forms on these agars will be discussed later.

One remarkable feature of these experiments was the ease with which *Sphacelotheca sorghi* could be cultured. Several of the smuts have been studied in culture at the Kansas Agricultural Experiment Station, but none seemed to be so easily cultured as *S. sorghi*. It proved to be relatively easy to secure cultures free from contamination and to maintain them without loss of vigor. The simplicity of the methods of culturing described above indicates the facility with which this organism may be handled in culture.

EXPERIMENTAL RESULTS

The fungus taken from wholly or partially smutted heads was grown separately on several artificial media. Constant differences in cultural characteristics between them have been noted during the time they have

been studied. These differences are sufficiently marked and constant to make it possible to distinguish the three strains studied as separate physiologic forms by cultural characteristics alone.

The cultural differences of the three forms of covered kernel smut from single chlamydospores when grown on carrot agar, potato-dextrose agar, and oatmeal agar are given in tables 1, 2, and 3, respectively. The differencens shown by cultures of the three forms growing on potato-dextrose agar are illustrated in figure 1. Color determinations were made

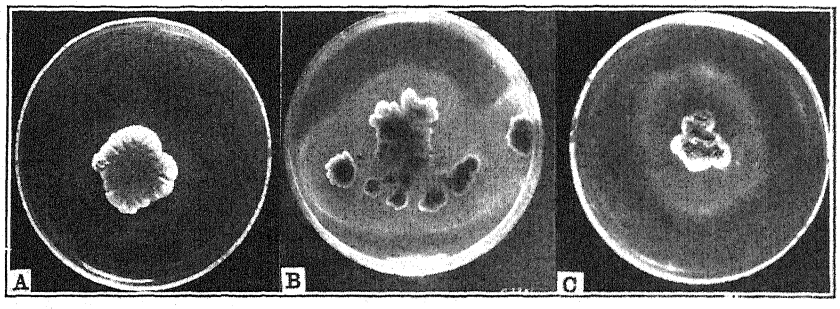


FIG. 1. Twelve-day cultures of three physiologic forms of *S. sorghi* grown on potato-dextrose agar at 80° F.
A. Physiologic form 1 from Pink kafir.
B. Physiologic form 2 from Dwarf Yellow milo.
C. Physiologic form 3 from feterita.

according to Ridgway's color standards and color nomenclature (7).

When grown on carrot agar the outstanding differences of these forms are as follows: Form 1 is characterized by an ivory-yellow color, and bacterioid consistency in young cultures. Later it becomes felted, the center usually being tufted in old cultures. Form 2 ranges from cinnamon-buff to cream-buff in color when young, turning to an umber color with age. The topography changes from beaded in young cultures to rather densely reticulate in fully developed cultures. Form 3 may be distinguished from the other two by its olive-buff color and its sparse spiny projections, which later disappear, the culture then taking on a beaded appearance.

When the cultures are grown on potato-dextrose agar, the colonies of form 1 have cream-buff centers with the remainder ivory-yellow. Their topography is relatively flat, either with or without reticulation. In form 2, the centers of the colonies are russet to cinnamon-brown and the remainder cream-buff in color, while their topography is rather flat with raised reticulations. In form 3, the colonies are pale olive-buff to dark olive-buff with very fine spiny projections and small beads.

When grown on oatmeal agar the outstanding color differences among these forms are as follows: Form 1 is cartridge-buff in the center and the

TABLE 1.—*Cultural characteristics of physiologic forms of Sphacelotheca sorghi on carrot agar. 12 days old*

Host	Form	Color	Topography	Surface	Consistency	Margin
Pink kafir (Bot. 1928 Row No. 23)	1	Ivory-yellow	Raised; center sparsely tufted; remainder reticulate	Waxy	Dry bacteroid	Lobate ridges; bacterioid
Milo (Bot. 1928 Row No. 43)	2	Cinnamon-buff to to Saccardo's umber	Raised; beaded to reticulate	do	do	Lobate; slightly beaded, bacterioid
Feterita (Bot. 1928 Row No. 21)	3	Olive-buff	Raised; portions of upright tufts; remainder beaded	do	do	Wavy; smooth with very fine striations, bacterioid

TABLE 2.—*Cultural characteristics of physiologic forms of Sphacelotheca sorghi on potato-dextrose agar. 12 days old*

Host	Form	Color	Topography	Surface	Consistency	Margin
Pink kafir (Bot. 1928 Row No. 23)	1	Cream-buff; margin ivory-yellow	Slightly raised; very finely reticulate	Waxy	Dry bacterioid	Wavy to lobate ridges, bacterioid
Milo (Bot. 1928 Row No. 43)	2	Center russet to cinnamon-brown; margin cream-buff	Relatively flat; raised reticulate ridges	do	do	Wavy, bacterioid
Feterita (Bot. 1928 Row No. 21)	3	Pale olive-buff	Slightly raised; beaded	do	do	Slightly wavy; mycelioid

TABLE 3.—*Cultural characteristics of physiologic forms of Sphacelotheca sorghi on oatmeal agar. 12 days old*

Host	Form	Color	Topography	Surface	Consistency	Margin
Pink kafir (Bot. 1928 Row No. 23)	1	Center cartridge-buff; remainder covered with a white felt	Flat	Center waxy; remainder dull	Center dry bacterioid; remainder myceloid	Entire and myceloid
Milo (Bot. 1928 Row No. 43)	2	Center russet; remainder white	do	do	do	Slightly wavy and myceloid
Feterita (Bot. 1928 Row No. 21)	3	Olive-buff with spots of russet; margin white	Flat; raised beads	Waxy; margin dull	Dry bacterioid; margin myceloid	Wavy, myceloid

remainder is covered with a white felt. Form 2 is umber to russet in the center and white elsewhere. Form 3 is olive-buff with spots of russet, and a white margin.

The rate of growth of the three forms varies with the media on which they are grown. The rate of growth of all three forms on oatmeal agar is about the same, but on carrot and potato-dextrose agar form 2 grows more rapidly than either form 1 or 3. On carrot and potato-dextrose agar form 1 usually has been a more rapid grower than form 3.

The formation of wedge-shape or fan-shape sectors in single and multi-chlamydospore cultures of form 1 has been mentioned. These also have been observed in one of the cultures of form 2. These sectors differed from the parent cultures in one or more characters, such as rate of growth, consistency, topography, or color. Most of them differed by a single character, but a few differed in two or more. In all cases such sectors were sufficiently different from the remainder of the cultures to be easily noticeable. Transfers were made from the margins of the sectors and new cultures developed from them. These have been transferred frequently and have maintained their distinguishing characteristics without change (Fig. 2).

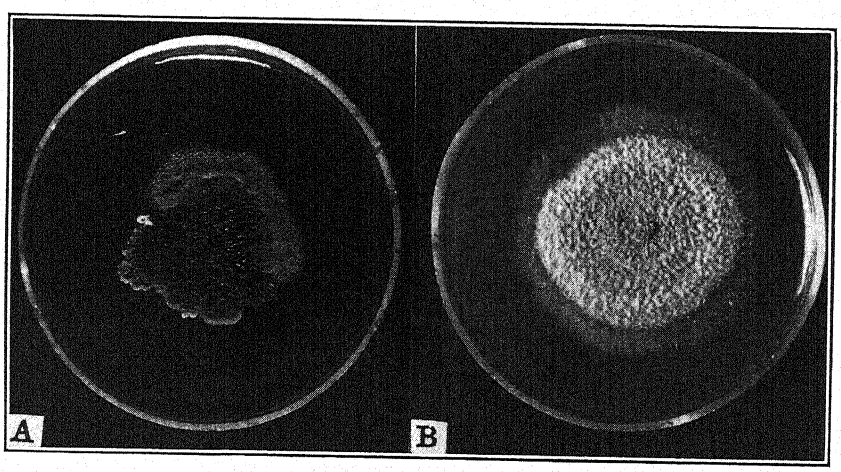


FIG. 2. Sectoring in physiologic form 2 of *S. sorghi* on carrot agar.
A. Main portion of culture bacterioid cinnamon-buff in color with a cream-buff margin. Sector mycelioid, gull-gray in color.
B. Culture grown from a marginal transfer from the sector shown in A.

Thus far only a limited number of monosporidial cultures have been made and no sectoring has been noted in them. In some cases two different types of cultures were obtained from separate monosporidial cultures of a single physiologic form. For example, bacterioid and mycelioid cultures were derived from monosporidial cultures of form 3. In all cases these cultures

were constant for their respective characteristics in subsequent generations.

Sectoring was of frequent occurrence in cultures of form 1, arising either from single or many chlamydospores. The phenomenon has not been observed in form 3 and only one case has been noted in form 2. Only a limited number of cultures has been studied thus far, however, and further studies may reveal them in all three physiologic forms. On the basis of the present experiments it seems that form 1 is relatively unstable, while forms 2 and 3 are very stable. This is of interest from a historical standpoint, as form 1 has long been known, whereas forms 2 and 3 have been observed only recently. Tisdale, Melchers, and Clemmer (11) discussed the possibility of hybridization of *S. sorghi* and *S. cruenta* (Kühn) Potter having given rise to a new form of sorghum smut. Leonian (5) has shown that many new types arise in Phytophthora by mutation, while Christensen (1) noted the same phenomenon for *Helminthosporium sativum* P., K., and B. Christensen and Stakman (2) and Stakman, Christensen, and Hanna (10) have demonstrated the formation of new forms of corn smut in culture by the same process. More recently Rodenhiser (8) noted mutations in *U. hordei* and *U. avenae* which differed from the parent cultures in color and rate of growth.

On the other hand, Stakman and Christensen (9) have shown that corn smut is heterothallic and that hyphal fusions of cultures of opposite sex were necessary before the fungus was able to fruit. Hanna (4) confirmed these observations for *U. zeae* and demonstrated the same phenomenon in *Sorosporium reilianum* Kühn (McAlp.). Dickinson (3) has also shown that cultures of different sex also frequently have strikingly different characteristics in culture.

Thus it seems that the sectoring noted in physiologic form 1 of *S. sorghi* may have been due either to mutation or to heterothallism. Further studies on these points are under way. The evidence at present points to heterothallism rather than to mutation, as the sectors observed arose in cultures from the diploid chlamydospores rather than the haploid sporidia. Although chlamydospores are uninucleate the single nucleus arises in the young spore cells by the fusion of two nuclei, according to Rawitscher (6). It should be possible, therefore, for sectors to arise by segregation or by the fusion of sporidia in the heterothallic culture.

SUMMARY

Three physiologic forms of covered kernel smut of sorghum are shown to be distinct in culture on various agar media. They differ in color, surface, consistency, margin, and rate of growth.

Physiologic form 1 attacks Pink kafir, but is unable to infect milo or feterita. Form 2 attacks milo to a moderate extent and Pink kafir severely,

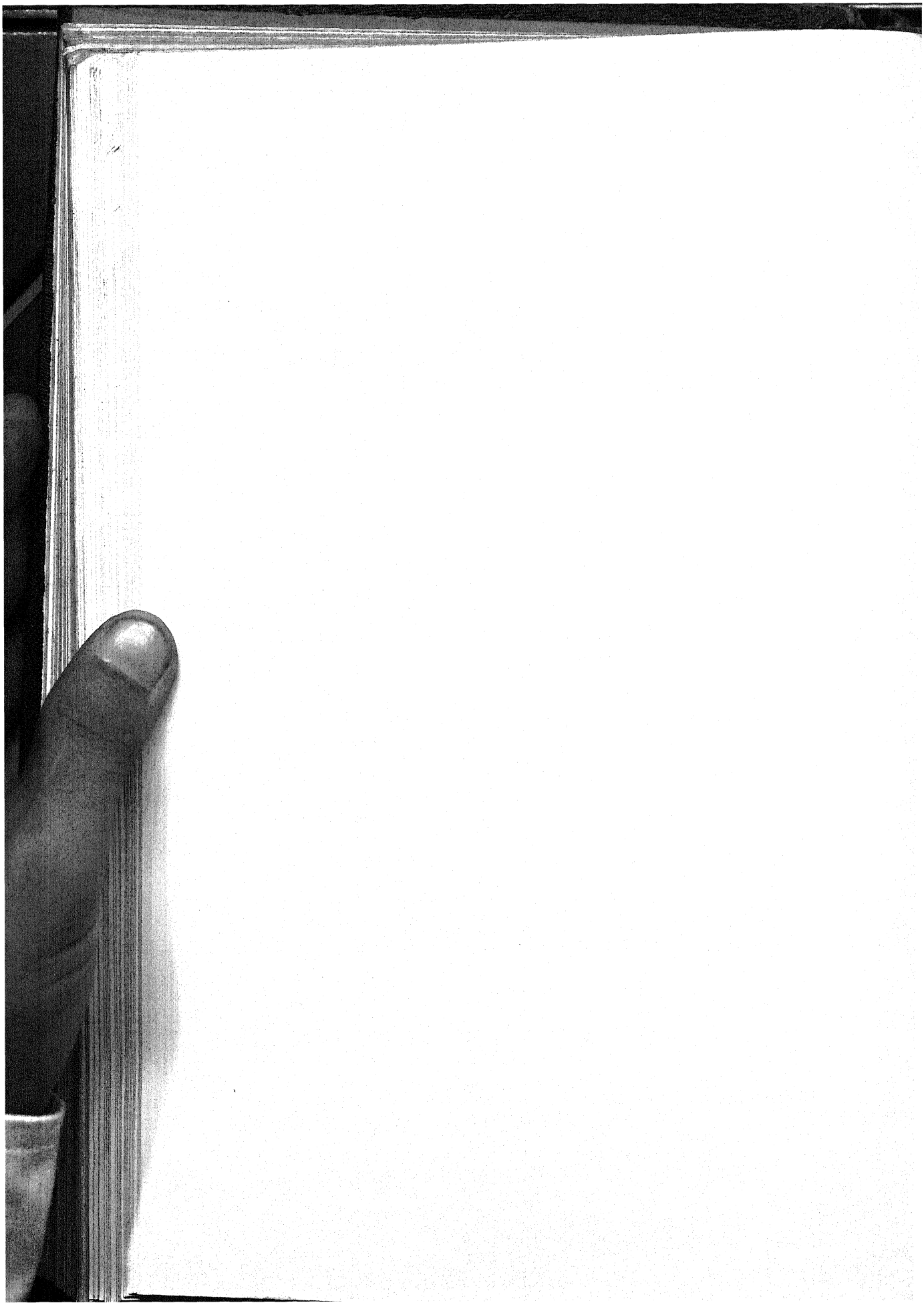
but is unable to attack feterita. Form 3 attacks feterita to a moderate extent and Pink kafir rather severely, but is unable to attack milo.

Multi- and single-chlamydospore cultures were made of all three forms and monosporidial cultures also were studied to a limited extent. Sectoring was observed rather frequently in cultures of form 1 arising from chlamydospores, but not in monosporidial cultures. The other forms seemed to be very stable, sectors appearing only in a single instance in form 2 and not at all in form 3.

The cultural characteristics seemed very stable in most cases, successive transfers and new cultures from the original inoculum yielding cultures with the same distinguishing characteristics.

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SOME MICROSCOPICAL STUDIES ON PENICILLIUM DECAY OF CITRUS[1]

L. J. KLOTZ[2]

Histological observations were made on fresh and paraffined sections of fruit decayed by *Penicillium digitatum* (Fr.) Sacc. and *P. italicum* C. Wehmer. Pieces of lemon and orange fruit, including both the diseased areas and the healthy, noninvaded regions, were killed and fixed in chrom-acetic-urea solution, embedded in paraffin, sectioned 6 to 15 μ in thickness, and stained with Magdala Red and Licht Grün according to the method of B. T. Dickson (2) or the Pianeze method of Vaughan (11). The latter combines the stains malachite green, acid fuchsin, and Martius Gelb in one solution. With both methods the host tissue is stained green and the mycelium pink to red. I obtained better staining and differentiation with the former method.

Although the types of decay produced, respectively, by these two fungi are macroscopically easily differentiated [Fawcett and Lee (3), pp. 353–362, fig. 112], in these studies no microscopical differences were detected in the method of attack of the two organisms. The hyphae were without definite haustoria and were distinctly intracellular as well as intercellular [Fig. 1, A, B, and D and compare with Smith (10)]. Some hyphae had very apparent constrictions where they penetrated the cell walls. They were tortuous, ramifying, and extremely variable in thickness, ranging from 3 μ to 17 μ in our material, as shown in figure 1, B and E. The large, densely-granular mycelial threads completely filled some of the host cells (Fig. 1, B and D). The fungi penetrated all cells comprising the albedo and flavedo, including the cells of the oil glands, but avoided the space containing the oil. Hyphae of both fungi invaded the region of the juice vesicles, but in the material used, taken at the advancing margin of the rot, they were usually found only in the vesicle tissue next to the albedo, that is, in the cells of the stalks of the juice vesicles. Advanced stages of the decayed tissue were not studied by means of the paraffin method, but it was found that individual juice vesicles taken from fruit decayed by *P. italicum* always contained mycelium. The cells of the expanded part of the vesicle containing the juice seemed the last to be invaded.

Epidermis stripped from the margin of the decayed portion of an orange attacked by *P. italicum* showed that, in fruiting, the hyphae of

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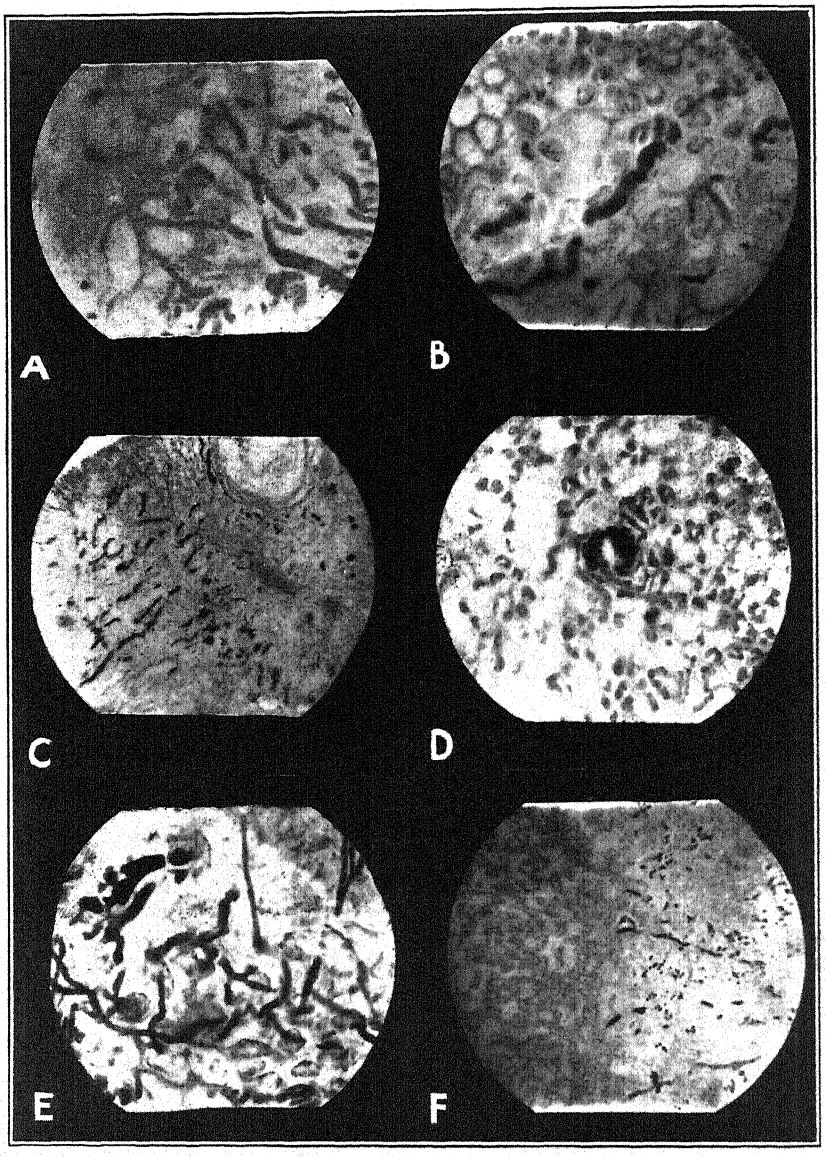


FIG. 1. Hyphae of Penicillia in rind of Citrus. A, B, C, E, F, in albedo. D, surface view of epidermis.

the rind collect substomatally as heavy-wall threads and crowd up through the surface, wedging and crowding apart the auxiliary cells, to form a fascicle of aerial hyphae upon which the condiophores and conidia ultimately appear (Fig. 1, D, and Fig. 2, A, B, and C). It is occasionally

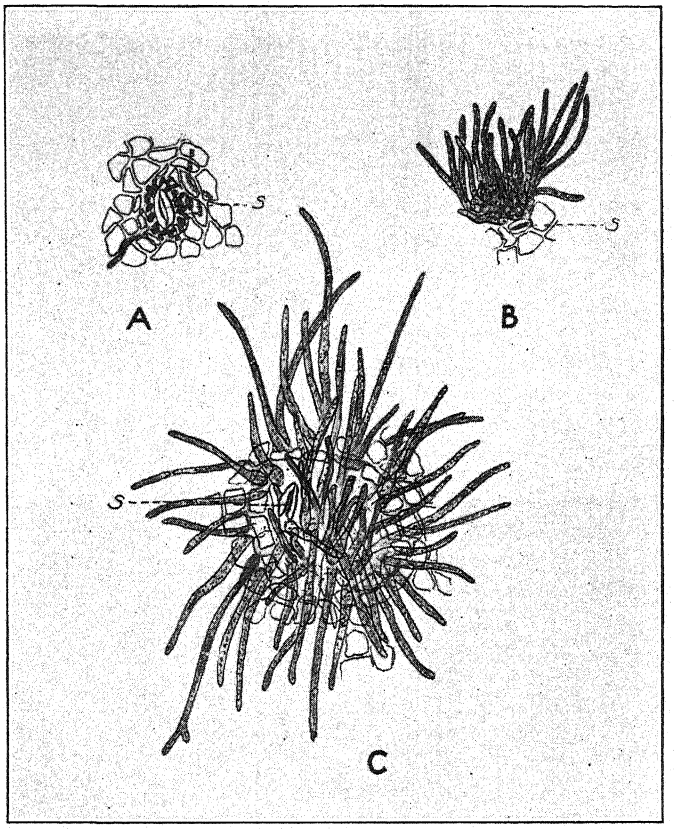


FIG. 2. Early stages of aerial fruiting hyphae on citrus. S—stoma.

possible to find the intact guard cells at the base of these fascicles. Sometimes the guard cells are crowded aside and are seen at the base of the periphery of the cluster of aerial hyphae (Fig. 2, B). In some instances the mycelial threads are seen penetrating the stomatal slit, but usually they surround the guard cells.

In these diseases little evidence was found of an affection of host tissue extending beyond the region actually invaded by the mycelium (Fig. 1, F). Johann (4, 5), working with a disease of corn seedlings caused by *Penicillium oxalicum,* found that the cells of the embryo and mesocotyl were apparently killed in advance of invasion by the mycelium. The causal fungus, a weak pathogene and chiefly intercellular, excretes an abundance of oxalic acid. The author found that this acid, placed on the surface of the seedlings, produced lesions similar to those caused by the fungus. Yellowing and desiccation of the leaves, which are typical

symptoms of the disease, could be simulated by placing corn seedlings in dilute solutions of the acid. Nobécourt (6), on the other hand, believed that the toxins or enzymes of *P. glaucum* and *Rhizopus nigricans*, which disorganize plants, were intracellular, since there is nothing in a carrot-broth medium after the fungi have grown in it that attacks carrot; whereas, an extract of the macerated fungus mats possessed remarkable power to disorganize the plant tissue. It would seem, however, that if the cultures had aged there would have been sufficient autolysis of the old hyphae to have released some of the macerating principle. With the citrus decays it was found that the albedo parenchyma in the region occupied by the mycelium of *P. digitatum* and a short distance beyond, probably never more than 3 mm. (300 μ), shows maceration and collapse and takes a less dense stain with the Licht Grün (Fig. 1, F). This region was even smaller in the decay caused by *P. italicum*, the mycelium being not more than 2 or 3 cells away from the deeply-stained region. This may indicate an advance in action, but it is too limited to be determined easily by isolation methods. I failed repeatedly to isolate the organisms from solid tissue just beyond the margin of decay, nor were they found in fresh microscopic mounts made from this region. On the other hand, they were always present in the tissue that showed even the slightest softening. Complete collapse of the host cells was seen and finally only traces of them were found in the advanced stages of decay, the space being occupied by the mycelium of the fungi. These findings are in contrast to those of Rushton (8).

The above observations indicate that the macerating substances are for the most part intracellular, as Nobécourt found with *P. glaucum*, and are secreted in limited quantity only and near the tips of the living, invading hyphae. They probably are liberated *en masse* from old, dead, autolyzing mycelium in the regions of advanced decay. To test these observations the following simple experiments were made. Mycelial mats of the fungi were dried with acetone and ether, followed by a temperature of 45° C. for 24 hours; then ground to a fine powder and covered with toluene. The toluene was finally driven off by exposure to a temperature of 45° C. This material was mixed with twice its volume of water and inserted in cylindrical holes made in the albedo of orange and pummelo. The softening produced was distinct but limited to the margin of the holes, extending but a short distance into the albedo. A remarkable thing to be noted here was that some portions of the fungi, spores or resistant mycelium, were living after the drying process, the grinding, and the treatment with toluene, provided the toluene was not allowed to remain in contact with the powder but was at once exposed to a temperature of 45° C. and thus gradually driven off. Material which had been treated for a week with

toluene had no living fungus. Extracts of the fungus powder were made by placing a gram of the material in 100 ml. of distilled water for one hour, shaking vigorously at 5–10 minute intervals, and then filtering the suspension by suction through a double thickness of sterile filter-paper. Five-ml. quantities of the filtrate were injected hypodermically into the albedo of orange and pummelo. Observation at the end of 16 hours revealed marked softening of the treated albedo, which closely resembled the decay caused by the living fungi. The area of maceration was outlined with India ink and was observed as time passed to extend beyond this demarcation. Control fruit injected with distilled water was not macerated.

Injections of 1 per cent oxalic-acid solution caused a maceration in pummelo and orange rind, the latter being firmer and more turgid than the former. The portion of the rind affected by the acid was depressed uniformly, making a smooth, rather firm area. The effect produced by the extract of the dried fungus differed from that of the chemical in being softer and less uniformly sunken and of a faintly brown color. Moreover, the extract, unlike that of *P. oxalicum* studied by Johann (5), gave no qualitative test for the oxalate ion. Neither distilled water nor a 1 per cent solution of ammonia (C. P. 28 per cent) caused an effect on orange or grapefruit detectable in 18 hours. After 48 hours the orange injected with ammonia showed a slight browning and a shallow, irregular sinking; the pummelo continued to be unaffected at the end of that period. However, the rind of fruit injected with a 5 per cent solution of ammonia acquired within 5 hours a sayal-brown to cinnamon-colored area [Ridgway (7)], which was much firmer and considerably darker than that caused by the fungus extract. It has been observed by Savastano and Fawcett (9) that oranges decayed by Penicillium species at high temperatures acquire a discoloration resembling that caused by the ammonia. The resemblance of ammonia injury to Phomopsis stem-end rot had been recorded by Bahgat (1). It is possible also that in the Penicillium decay the ammonia liberated by the desaminases and desamidases of the fungi is in part responsible for the injury.

SUMMARY

To sum up, it was found, among other things, that *Penicillium digitatum* and *P. italicum*, important organisms in the decay of Citrus fruits, grow inter- and intracellularly in the albedo parenchyma, flavedo, and epidermis and finally invade the juice vesicles.

The hyphae of these fungi, which are very variable in width, collect substomatally and thrust aerial fruiting hyphae through the epidermis in the neighborhood of the auxiliary and guard cells. The mycelium exhibits but a very limited effect in advance of invasion.

The nature of the decay by the living fungus was compared and contrasted with the softening caused by injections into the albedo of solutions of oxalic acid and ammonia and of an extract of the fungi.

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FURTHER STUDIES OF PRIVET ANTHRACNOSE

A. J. Mix

DISTRIBUTION OF THE DISEASE

In an earlier paper (4) on privet anthracnose, caused by *Glomerella cingulata* (Stonem.) Spaulding and von Schrenk, which included an account of the history and distribution of the disease, it was stated that, although the fungus *G. cingulata* had been the subject of much investigation, there seemed to have been no mention of privet anthracnose in the literature later than the account given by Atkinson (2) in 1892. This statement was due to the fact that a reference by Clinton (3) had been overlooked. Clinton records the occurrence of the disease in Connecticut in 1913 and also mentions the occurrence of basal cankers and the consequent death of the plant.

Privet anthracnose seems to be more widely distributed than the references cited above would indicate. Anderson and others (1), in their Check List of Diseases of Economic Plants in the United States, mention this disease as "widespread east of the Great Plains." Its destructive occurrence within the Great Plains area has been previously reported (4).

OCCURRENCE OF THE DISEASE ON A NEW PRIVET

In all of the above-mentioned accounts of privet anthracnose, the host species concerned has been *Ligustrum vulgare* L. In the fall of 1925, a letter was received from Dr. H. W. Anderson describing the occurrence of the anthracnose in an Illinois nursery. It had caused severe loss in some plantings of a new privet called Lodense. Some Lodense privet plants which were received from a nursery in 1927 and planted on the campus of the University of Kansas developed in the summer of 1929 typical twig blight and cankers of the anthracnose. *Glomerella cingulata* was isolated from these blighted twigs and cankers. Beyond these instances, nothing further is known of the occurrence of the anthracnose on Lodense privet, nor, for that matter, of the extent to which the Lodense privet has come into cultivation.

THE LODENSE PRIVET

The Lodense privet was introduced to the trade by the Henry Kohankie and Son Ornamental Nurseries, Painesville, Ohio. Mr. H. J. Kohankie states that they still have the original plant and that it was found among a lot of seedlings of European privet which they imported from France some years ago. It is his opinion that it is a "sport" of *Ligustrum vulgare.*

It has not been possible to determine the botanical relationships of this privet. It is not recognized in botanical literature under the name Lodense. In the general appearance of its foliage, flowers, and fruit it resembles *L. vulgare,* but in comparison with that species possesses a decidedly compact, dwarf habit. Miss Amelia Babcock, in the course of some comparative morphological and anatomical studies of species of *Ligustrum,* has found the Lodense privet to differ as widely from *L. vulgare* as did several other recognized and named species. On the basis of her work it seems undoubtedly distinct from *L. vulgare, L. ibota, L. amurense, L. ovalifolium, L. ibolium, L. quihoui,* and *L. regelianum.* (Miss Babcock's work is reported in an unpublished thesis of the University of Kansas.)

PURPOSE OF EXPERIMENTS

The inoculation experiments, to be described below, were undertaken with two purposes in mind. The first was to test the susceptibility of the Lodense privet to anthracnose. This seemed worth while since the inoculation experiments reported earlier (4) were performed with several species of privet, of which only *Ligustrum vulgare* proved susceptible.

The second purpose was to determine whether *Glomerella cingulata* from apple could attack privet, and vice versa. Shear and Wood (6) have shown that this fungus is morphologically and physiologically the same on many different hosts, but among the large number of cross-inoculation experiments which they performed they did not include any between privet and apple. Since susceptible privets may be planted in localities where apples are grown and where bitter rot occurs, it seemed worth while to determine whether the fungus can pass from the one host to the other. In the earlier inoculation work (4) it was learned that *G. cingulata* from privet could cause decay in ripe apple fruits. A few successful inoculations of privet branches with *G. cingulata* from apple fruit were also reported. The inoculations described below were intended to secure additional data on this latter point.

INOCULATIONS

Plants of Lodense and of European privet were secured in the spring of 1927. These appeared to be about two years old. Some of them were planted in the greenhouse and a few out of doors. Additional plants were secured by making cuttings from the branches of the older plants. Inoculations were performed on the older plants immediately and on the younger plants during their first season of growth. Since there proved to be no difference in susceptibility between plants of different ages, except that the younger plants died more quickly, nor between plants growing in the greenhouse and out of doors, the results have been summarized

without regard to age or location of the inoculated plants. These results are recorded in tables 1, 2, and 3.

TABLE 1.—*Results of inoculating privet plants with Glomerella cingulata by means of incisions into main stem below ground*

	Kind of privet	Source of culture	Number of incisions	Number of infections
Inoculated	Vulgare	Privet	20	19
"	"	Apple	28	4
Controls	"	.....	7	0
Inoculated	Lodense	Privet	7	6
"	"	Apple	6	4
Controls	"	.....	4	0

TABLE 2.—*Results of inoculating privet plants with Glomerella cingulata by means of incisions into branches*

	Kind of privet	Source of culture	Number of incisions	Number of infections
Inoculated	Vulgare	Privet	10	10
"	"	Apple	13	1
Controls	"	.....	11	0
Inoculated	Lodense	Privet	3	3
"	"	Apple	11	10
Controls	"	.....	11	0

TABLE 3.—*Results of inoculating privet plants with Glomerella cingulata by spraying with spore suspensions*

	Kind of privet	Source of culture	Number of plants	Number of plants becoming infected
Inoculated	Vulgare	Privet	10	10
"	"	Apple	3	0
Controls	"	.....	3	0
Inoculated	Lodense	Privet	2	2
"	"	Apple	8	3
Controls	"	.....	3	0

Two methods of inoculation were used: 1. Incisions into bark and wood (Tables 1 and 2), with insertion of spores and mycelium from culture, the wounds being left unprotected; 2. Spraying of foliage and stems with sterilized-water suspensions of conidia from culture (Table 3), the plants being subsequently covered with bell jars for 48 hours. Controls consisted

in uninoculated and unprotected incisions and in spraying plants with sterilized water and covering them as above.

Two cultures of *Glomerella cingulata* which had been isolated from European privet were used for these inoculations, and three cultures from apple. One of these apple cultures had been isolated from fruit by Dr. H. W. Anderson, and the other two were isolated from Willow Twig apple fruits affected with bitter rot, also kindly furnished by Dr. Anderson. Since no significant differences in pathogenicity seemed to exist between different cultures from the same host, the culture numbers are not included in the tables.

Infection was considered to have occurred when an incision was found unhealed and a considerable portion of the wood of the stem was discolored and dead, or, in the case of spray inoculations, when definite leaf spots and stem cankers (always a considerable number on one plant) had developed. *Glomerella cingulata* was recovered by reisolation from a number of inoculated Lodense and European plants.

CONCLUSIONS FROM INOCULATIONS

A study of the results shown in these tables seems to justify the following conclusions:

1. Lodense privet appears fully as susceptible to infection by *Glomerella cingulata* as does European privet and it should not, therefore, be recommended for planting as a hedge plant in localities where the fungus occurs. It may be emphasized in this connection that for use in ornamental hedges a plant needs to be highly resistant, if not entirely immune from disease.

2. *Glomerella cingulata* from apple is capable of causing infection on Lodense and European privet, even if somewhat less readily than the same fungus from privet. A locality in which apple bitter rot occurs would therefore be unsafe for these privets.

It may be further noted that Lodense privet seems to be more readily attacked by *G. cingulata* from apple than is European privet.

INOCULATIONS OF APPLE BRANCHES

Inoculations of apple branches with *Glomerella cingulata* previously reported (4) resulted in no infections. A new series of such inoculations with cultures of *G. cingulata* from both privet and apple was made in 1927. These were wound inoculations made by means of incisions into one-year old and two-year old branches of young Ben Davis, Jonathan, Northwestern, and seedling apple trees. From 20 to 30 such inoculations were made with each variety. No infection resulted.

Von Schrenk and Spaulding (5) are apparently the only investigators who have successfully inoculated apple branches with *Glomerella cingulata.*

In their experiments the wounds were covered with grafting wax after inoculation, while in those reported here the wounds were left unprotected. This may account for the difference in results.

SUMMARY

Anthracnose, caused by *Glomerella cingulata* (Stonem.) Spaulding and von Schrenk, besides being widely distributed in the United States on European privet, occurs also on a new privet called Lodense.

The botanical classification of the Lodense privet is not known, but it seems to be distinct from *Ligustrum vulgare* and from several of the other commonly grown species of privet.

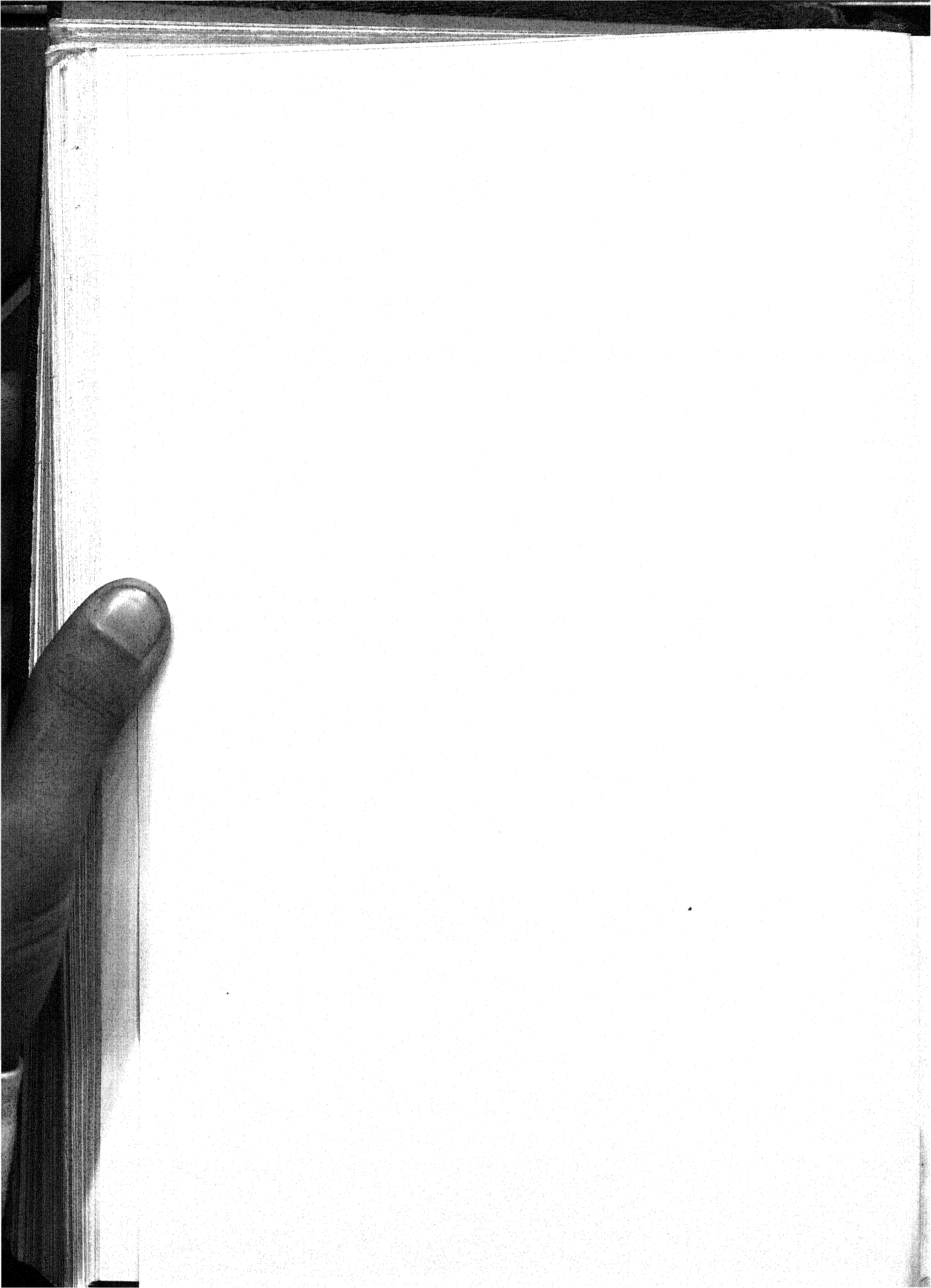
Inoculation experiments have shown that *Glomerella cingulata* from privet and from apple can cause infection on both European and Lodense privet.

A series of inoculations of the young branches of apple trees with cultures of *G. cingulata* from both privet and apple resulted in no infection.

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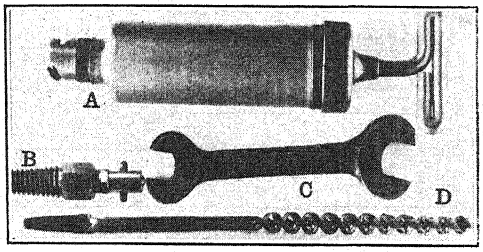
A SIMPLE TREE INJECTOR

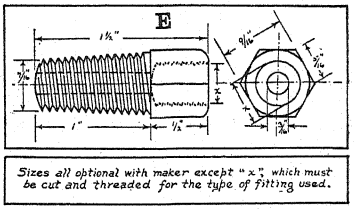
RUSH P. MARSHALL

Interest has been shown in a simple method of tree injection demonstrated by the writer at a conference of shade tree workers held in Stamford, Connecticut, in the summer of 1927. Believing that pathologists who work with tree species will find the method useful for rapid injection of small quantities of material, the apparatus used is here briefly described.

It consists in the application of the automobile grease gun to the injection of trees. With the exception of a single fitting, the parts may be borrowed from the automobile and laboratory tool box or purchased at small cost from the nearest hardware store.

This particular fitting (Fig. 1, B and E) can be turned out in a few





minutes by a machinist if the experimenter himself has not the facilities for making it. It is a brass tube threaded at one end to receive an Alemite bearing and cut at the other to form a wood screw. A working drawing is supplied. The dimensions given are those of the fitting used by the writer, but they are optional.

A number of guns of the regular and "Zerk" types were experimented with. Of those tested, the small, regulation, Alemite gun, sold for use with 600 W. oil, was found most suitable for the present purpose. The complete outfit, with the exception of a brace, is easily carried in the pocket (Fig. 1; A, B, C, and D); it has no hose to develop leaking connections; it is said to give a pressure of 500 pounds to the square inch; it will hold two ounces of material.

These guns are designed for use with heavy oil or light grease, and no difficulty is experienced in injecting trees with substances which approximate their consistency. The apparatus is less practical for the injection of water solutions, and, if they are to be used, care is required. The leather plunger must be in good order and neither worn nor folded. If new, it

should be softened with some such dressing as neatsfoot oil or Viscol and then coated with cup grease. When larger guns with hoses are used, it is generally necessary to pack the hose connections with spray-pump packing to prevent leakage. Guns of the larger type inject heavy materials readily, but are practically worthless for aqueous solutions.

The process of injecting the tree is simple. Using a brace with a bit the same size as the small end of the wood screw, a hole is bored nearly through the trunk. The mouth of the opening is then faced with a countersink, and the wood screw of the fitting into which the Alemite bearing has previously been screwed is turned into the wood with the help of a wrench. The remainder of the operation consists in turning down the handle as in greasing a car, except that in using water solutions it is necessary to make the first several turns very quickly in order to develop sufficient pressure to create good contact between the nozzle of the gun and the bearing. The rapidity with which the material may be forced into the wood varies greatly with the tree, the substance used, and the time of application. In general, the contents of a two-ounce gun can be injected in from five to fifteen minutes.

Following use, the gun is easily cleaned. It may be sterilized with boiling water, but sterilization by dry heat is to be avoided on account of its effect in drying out the leather plunger.

The apparatus here described has been used in attempts to force preservative materials into pruning wounds, to inoculate trees with fungous cultures, and to make experimental injections to control borers. It has worked effectively for substances the consistency of which is approximately that of grease or heavy oil, and poorly for aqueous solutions. Since the rapidity with which the material is forced into the trunk under high pressure does not give time for any great degree of distribution during the process of injection, the amount of material which can be forced in is limited. For this reason, the grease gun cannot supplant injection apparatus in ordinary use. It is valuable where the rapid injection of not more than several ounces of material to a hole is involved and it will inject many materials of a consistency too thick to permit of injection by usual methods.

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PHYTOPATHOLOGICAL NOTES

A blight of flowering almond, Prunus glandulosa Thunb.—Diseased specimens of the dwarf flowering cherry or flowering almond, *Prunus glandulosa* Thunb., have been observed at Lawrence, Kansas, during the past three seasons. In each of these years the disease was evident in late spring and early summer, following a rather prolonged period of wet weather. Blossoms, leaves, and twigs turned brown and died and were frequently covered with the conidial tufts of a Monilia. In some cases branch cankers developed around the bases of affected twigs.

From such blighted leaves and twigs a fungus was readily isolated. It proved to be the Monilia stage of the common brown-rot fungus, *Sclerotinia fructicola* (Wint.) Rehm. In cultural characters and in morphology of mycelium and conidia formed in culture it appeared closely similar to, or identical with, strains of the same fungus isolated from cherry and apple fruits. Sound fruits of peach, plum, and apple developed typical brown rot when inoculated with the Monilia from cherry, apple, and flowering almond. Shoots of flowering almond with opening blossoms were removed, sprayed with spore suspensions prepared from cultures of the three strains mentioned above, and kept, with their lower ends in water, under bell-jars lined with moist filter-paper. Blossom and leaf blight resulted in all cases. The fungus was reisolated from each set of twigs.

So far as can be determined, the disease has been reported previously only once. W. C. Sturgis (Connecticut Agr. Exp. Sta. Rept. 1898: 261–267. 1899), in describing an outbreak of brown-rot blossoms and twig blight in Connecticut in 1898, states: "The trouble . . . was very common also on the ornamental shrub known as the Double Flowering Almond." I collected specimens of the disease (from which the fungus was later isolated) at Collinsville, Connecticut, in the summer of 1928.

The disease does not appear to be very destructive in this locality. It has been kept in check during the past three seasons by careful pruning out of affected branches, and such pruning has left the shrubs in thrifty condition.—A. J. Mix, University of Kansas, Lawrence, Kans.

Brown-rot leaf and twig blight following peach-leaf curl.—The usual account of the peach-leaf-curl disease states that the curled leaves eventually die and fall from the tree. In reporting (Mix, Phytopath. 14: 217–233. 1924) some studies of *Exoascus deformans,* I called attention to the fact that, after ascospore dispersal has occurred, curled leaves are commonly invaded by other fungi. These fungi accomplish the final destruction of the leaf, but have never been observed to pass from the curled portion of the leaf into the adjacent healthy portion. For this reason no particular

attention has been paid to their identity, but species of Alternaria and Fusarium have been observed and a species of Cladosporium is common.

In June, 1928, while collecting peach-leaf curl in unsprayed orchards in the vicinity of New Haven, Connecticut, I noticed that nearly all of the curled leaves and shoots were being destroyed by the brown-rot fungus, *Sclerotinia fructicola* (Wint.) Rehm. In contrast to the fungi mentioned above, this fungus was growing from the curled portions of leaves and twigs into the adjacent healthy portions. In some cases healthy leaves in contact with curled leaves were being invaded by the brown-rot fungus. In short, brown-rot leaf and twig blight were abundant in these orchards and were at this time confined exclusively to leaves and twigs previously infected by the leaf-curl fungus. Peach fruits were apparently too young to be susceptible to brown rot; at any rate, no diseased fruits were found.

It was clear that in this season and in this locality the establishment of the brown-rot fungus in peach orchards had been aided by the previous establishment of the curl fungus. It is not known how common such an occurrence may be. It has not been observed under the less humid conditions of eastern Kansas. It seems, however, that here is an added reason for spraying peach trees to prevent peach-leaf curl.—A. J. MIX, University of Kansas, Lawrence, Kans.

PHYTOPATHOLOGY

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DISEASE AND CLIMATE AS PERTAINING TO THE FLORIDA AND MAINE POTATO SECTIONS

L. O. GRATZ

Approximately 26,000 acres of Irish potatoes are planted in Florida annually.[1] Over 19,000 acres of this total are planted in the Hastings area, which consists of a part of St. Johns, Putnam, Flagler, and Clay counties. Usually about 120,000 eleven-peck bags of State certified seed potatoes, valued at a half million dollars, are imported every year into Florida from Aroostook County, Maine. This amount of seed plants almost the entire acreage in the potato section proper.

The majority of the disease troubles of white potatoes are seed-borne. It is evident, other things being equal, that the Florida fields should exhibit symptoms of the same diseases as those observed in the Maine fields which produce the seed stock for this southern planting.

Careful field observations for 5 years, both in Florida and Maine, beginning in 1924 and ending in 1928, have demonstrated that some of the same troubles are found in both sections. Among the common diseases found in both States are late blight (*Phytophthora infestans* (Mont.) de Bary), early blight (*Alternaria solani* (E. & M.) J. & G.), rhizoctonosis (*Corticium vagum* B. & C.), scab (*Actinomyces scabies* (Thax.) Güssow), blackleg (*Bacillus atrosepticus* van Hall), and the various virus diseases (causes not determined). Some of these diseases do not produce such severe injury in Florida as in Maine. In the Hastings area, for example, scab and rhizoctonosis are not a commercial problem. Verticillium wilt (*Verticillium albo-atrum* Reinke and Berth.) is very prevalent during some seasons in Maine but has not been observed in Florida, and bacterial wilt (*Bacterium solanacearum* EFS.) is unknown in Maine but, at times, is very destructive in the southern potato sections.

These differences and the fact that, from the standpoint of the southern grower, the strategic point for the control of some of these diseases is in the seed-stock fields of the North have led to extensive observations in both sections, as reported herein.

[1] Average for 5 years, 1924–1928, as tabulated in U. S. Dept. of Agriculture, Crops and Markets, April, 1929, p. 123.

REVIEW OF LITERATURE

The relationship of environmental factors to the occurrence and severity of plant diseases has been recognized and studied for many years both by laymen and technical workers. Much experimental evidence to establish the exact facts regarding these relationships for plant pathogenes of all kinds has been accumulated both in this and other countries. Much work of this nature has been done in the laboratories of the Department of Plant Pathology at the Wisconsin Agricultural Experiment Station. An extensive summary, giving the results of experiments extending over a decade, with a complete citation of literature, is given by Jones, Johnson, and Dickson in their research bulletin No. 71 (9). Therefore, such a detailed review is unnecessary here.

Many field observations, confirmed by careful experimentation, have revealed that frequently small differences in mean temperature cause either slight attacks or severe epiphytotics, as the case may be. Such observations have likewise demonstrated that the geographic limitation of certain disease organisms may be due mostly to temperature differences. This is well illustrated in the above researches, as typified by cabbage yellows, flax wilt, rhizoctonosis of potatoes, and other diseases.

OBSERVATIONS AND INVESTIGATIONS

Climatological Data. Knowing that differences of environment cause variations in the severity of disease and observing that these differences in severity exist when Maine and Florida conditions are studied comparatively, the first step necessary to obtain more definite information concerning the relation of environment and potato diseases was to investigate climatic conditions during the respective growing seasons. Detailed weather records of these two sections were not readily available from previous publications. However, original data of the local weather observers were accessible in the Hastings belt, at Federal Point, Florida,[2] and at Aroostook Farm, Presque Isle, Maine. These figures were obtained and summarized. The records of the Florida observer date back 35 years, while those of Aroostook County cover a period of only 15 years. In order to make such climatological data for these two areas more readily available and of value for other investigations they are given here in complete detail (Tables 1–5; Figs. 1–3). From these original observations daily and monthly averages for the respective growing seasons were prepared for a period of 10 consecutive years both for Florida and Maine (Tables 1–3). In order that a clearer comparison may be made of the temperatures during the corresponding periods of the growing season the secular trends of the daily means were determined for both

[2] In the potato area 4 miles northwest of Hastings village.

TABLE 1.—*Daily average temperatures during the ten-year periods 1917–1926 and 1916–1926*[2] *for the potato-growing seasons at Federal Point, Florida,*[1] *and Aroostook Farm, Presque Isle, Maine, respectively*

FLORIDA				MAINE				FLORIDA				MAINE			
Date	Max.	Min.	Mean	Date	Max.	Min.	Mean	Date	Max.	Min.	Mean	Date	Max.	Min.	Mean
Jan. 15	67.0	45.4	56.2	May 15	60.4	35.6	48.0	Mar. 20	76.3	56.8	66.6	July 18	79.1	54.1	66.6
16	68.2	46.5	57.4	16	59.2	35.7	47.5	21	78.1	55.7	66.9	19	80.4	58.6	69.5
17	67.6	53.1	60.4	17	65.9	38.8	52.4	22	77.0	53.5	65.3	20	80.9	56.8	68.9
18	66.6	49.6	58.1	18	66.6	40.3	53.5	23	78.1	54.2	66.2	21	79.1	56.8	68.0
19	70.5	48.3	59.4	19	65.6	39.8	52.7	24	76.2	54.9	65.6	22	80.2	53.8	67.0
20	74.3	47.6	61.0	20	66.6	39.8	53.2	25	79.3	55.3	67.3	23	76.5	55.9	66.2
21	73.4	48.8	61.1	21	64.6	41.4	53.0	26	80.6	55.2	67.9	24	77.2	54.3	65.8
22	73.1	52.0	62.6	22	62.8	40.8	51.8	27	80.5	58.1	69.3	25	76.5	54.1	65.3
23	69.9	51.4	60.7	23	60.0	41.5	50.8	28	78.2	56.1	67.2	26	75.9	52.5	64.2
24	67.8	50.6	59.2	24	63.3	34.6	49.0	29	76.7	56.1	66.4	27	78.3	51.6	65.0
25	68.1	51.7	59.9	25	64.4	39.3	51.9	30	77.4	57.6	67.5	28	74.6	52.5	63.6
26	65.9	46.5	56.2	26	62.0	40.4	51.2	31	79.7	54.3	67.0	29	75.7	51.4	63.6
27	67.3	47.1	57.2	27	63.3	38.3	50.8	Apr. 1	77.1	55.9	66.5	30	75.9	53.7	64.8
28	69.3	51.0	60.2	28	68.3	39.1	53.7	2	76.8	52.5	64.7	31	77.6	56.2	66.9
29	69.1	50.1	59.6	29	69.3	41.5	55.4	3	79.1	53.2	66.2	Aug. 1	77.1	55.0	66.2
30	69.6	50.9	60.3	30	64.5	40.9	52.7	4	80.0	57.9	69.0	2	78.2	51.9	65.1
31	69.3	52.2	60.8	31	68.7	41.5	55.1	5	78.6	58.8	68.7	3	77.3	52.8	65.1
Feb. 1	70.0	49.6	59.8	June 1	71.9	43.3	57.6	6	77.4	57.0	67.2	4	77.1	49.6	63.4
2	67.3	49.8	58.6	2	68.7	41.6	55.2	7	78.9	56.0	67.5	5	77.7	49.7	63.7
3	65.9	46.4	56.2	3	71.8	46.1	59.0	8	81.4	56.0	68.7	6	78.8	52.2	65.5
4	67.1	48.6	57.9	4	74.8	50.6	62.7	9	79.5	56.8	68.2	7	73.2	57.8	65.5
5	67.2	49.7	58.5	5	74.9	48.9	61.9	10	78.5	58.3	68.4	8	74.3	56.9	65.6
6	67.4	46.0	56.7	6	73.6	47.4	60.5	11	77.8	54.9	66.4	9	75.6	52.7	60.0
7	69.0	44.1	56.6	7	73.1	45.0	59.1	12	79.5	55.7	67.6	10	76.5	53.0	60.4
8	69.0	45.3	57.2	8	71.2	49.4	60.3	13	81.1	57.5	69.3	11	77.5	54.1	65.8
9	70.7	46.9	58.8	9	70.0	45.9	58.0	14	79.8	56.2	68.0	12	75.6	51.5	63.6
10	71.0	44.9	58.0	10	70.2	46.5	58.4	15	81.0	58.0	69.5	13	75.3	54.5	64.9
11	71.2	44.9	58.1	11	68.0	46.8	57.4	16	81.0	59.8	70.4	14	75.3	53.6	64.5
12	71.0	46.8	58.9	12	68.8	47.8	58.3	17	83.0	59.5	71.3	15	73.7	51.9	62.8
13	72.7	48.6	60.7	13	72.7	45.8	59.3	18	82.7	58.8	70.8	16	78.4	53.9	66.2
14	74.7	50.3	62.5	14	74.2	47.6	60.9	19	82.3	56.8	69.6	17	76.5	53.0	64.7
15	75.1	50.4	62.8	15	71.6	47.9	59.8	20	83.5	55.6	69.6	18	73.5	49.8	61.7

[1] Florida observations taken at Federal Point, Florida, and Maine observations taken at Aroostook Farm, Presque Isle, Maine.
[2] Data for 1918 missing.

TABLE 1—(*Continued*)

FLORIDA			
Date	Max.	Min.	Mean
Feb. 16	70.7	45.7	58.2
17	69.6	47.4	58.5
18	70.6	51.5	61.1
19	71.4	49.8	60.6
20	72.4	50.3	61.4
21	73.5	50.8	62.2
22	74.3	49.2	61.8
23	74.5	50.9	67.7
24	75.5	52.0	63.8
25	75.0	51.9	63.5
26	74.0	52.2	63.1
27	72.5	51.8	62.2
28	73.7	53.4	63.6
Mar. 1	74.7	53.2	64.0
2	73.7	51.1	62.4
3	74.9	48.9	61.9
4	74.2	50.4	62.3
5	74.6	49.8	62.2
6	72.9	50.2	61.6
7	74.0	53.0	63.5
8	73.1	48.6	60.9
9	74.8	48.6	61.7
10	76.4	52.7	64.6
11	76.4	52.4	64.4
12	78.0	51.8	64.9
13	77.4	50.9	64.2
14	74.4	51.2	62.8
15	75.9	52.1	69.0
16	77.0	55.2	66.1
17	79.7	56.0	67.9
18	76.5	57.5	67.0
19	79.4	58.4	68.9

MAINE			
Date	Max.	Min.	Mean
June 16	68.2	45.7	57.0
17	67.3	46.5	56.9
18	67.3	46.6	57.0
19	71.3	49.8	60.6
20	74.2	47.8	61.0
21	73.3	50.2	61.8
22	71.6	49.0	60.3
23	74.9	47.6	61.3
24	75.8	49.1	62.5
25	75.7	49.1	62.4
26	77.3	50.1	63.7
27	76.1	50.0	63.1
28	74.3	53.4	63.9
29	70.9	51.3	61.1
30	73.6	53.0	63.3
July 1	74.1	51.1	62.6
2	75.4	51.6	63.5
3	76.5	51.8	64.2
4	75.7	53.2	64.5
5	76.4	51.9	64.2
6	78.8	50.7	64.8
7	78.8	54.8	66.8
8	79.9	54.4	67.2
9	77.1	50.6	63.9
10	77.1	52.3	64.7
11	77.8	53.2	65.5
12	79.7	55.5	67.6
13	79.2	55.4	67.3
14	78.0	53.5	65.8
15	80.5	55.9	68.2
16	76.3	54.8	65.6
17	77.4	55.4	66.4

FLORIDA			
Date	Max.	Min.	Mean
Apr. 21	84.4	58.7	71.6
22	84.1	59.3	71.7
23	84.1	60.0	72.1
24	83.7	58.7	71.2
25	83.4	57.1	70.3
26	85.6	61.6	73.6
27	82.2	60.5	71.4
28	86.1	56.4	71.3
29	85.4	59.2	72.3
30	83.5	59.4	71.5
May 1	82.8	59.4	71.1
2	83.7	60.7	72.2
3	80.6	60.1	70.4
4	84.2	58.9	71.6
5	81.9	59.4	70.7
6	83.2	60.1	71.7
7	84.8	59.6	72.2
8	85.3	60.7	73.0
9	84.7	58.6	71.7
10	86.1	58.9	72.5
11	87.5	60.9	74.2
12	87.5	61.1	74.3
13	88.0	64.2	76.1
14	85.5	63.9	74.7
15	84.3	63.2	73.8
16	84.3	62.1	73.2
17	84.3	63.1	73.7
18	85.3	63.2	74.3
19	85.4	63.4	74.4
20	87.0	63.4	75.2
21	85.6	63.9	74.8
22	86.5	64.3	75.4

MAINE			
Date	Max.	Min.	Mean
Aug. 19	75.0	52.1	63.6
20	74.7	51.0	62.9
21	70.2	49.4	59.8
22	71.9	47.5	59.7
23	73.5	52.2	62.9
24	73.2	54.2	63.7
25	74.9	54.0	64.5
26	70.8	52.5	61.7
27	72.1	49.7	60.9
28	72.8	51.3	62.1
29	73.0	52.8	62.9
30	73.5	53.2	63.4
31	71.4	50.9	61.2
Sept. 1	71.1	46.5	58.8
2	69.2	49.7	59.5
3	68.2	47.7	58.0
4	66.2	44.6	55.4
5	69.1	39.7	54.4
6	63.6	43.0	53.3
7	66.5	43.3	52.9
8	67.7	43.6	55.7
9	69.4	43.4	56.4
10	64.0	45.4	54.7
11	66.4	45.5	56.0
12	64.4	47.8	56.1
13	65.8	51.2	58.5
14	63.3	45.5	54.4
15	62.9	40.4	51.7
16	63.0	38.5	50.8
17	64.4	41.4	52.9
18	64.4	41.2	52.8
19	64.6	39.6	52.1

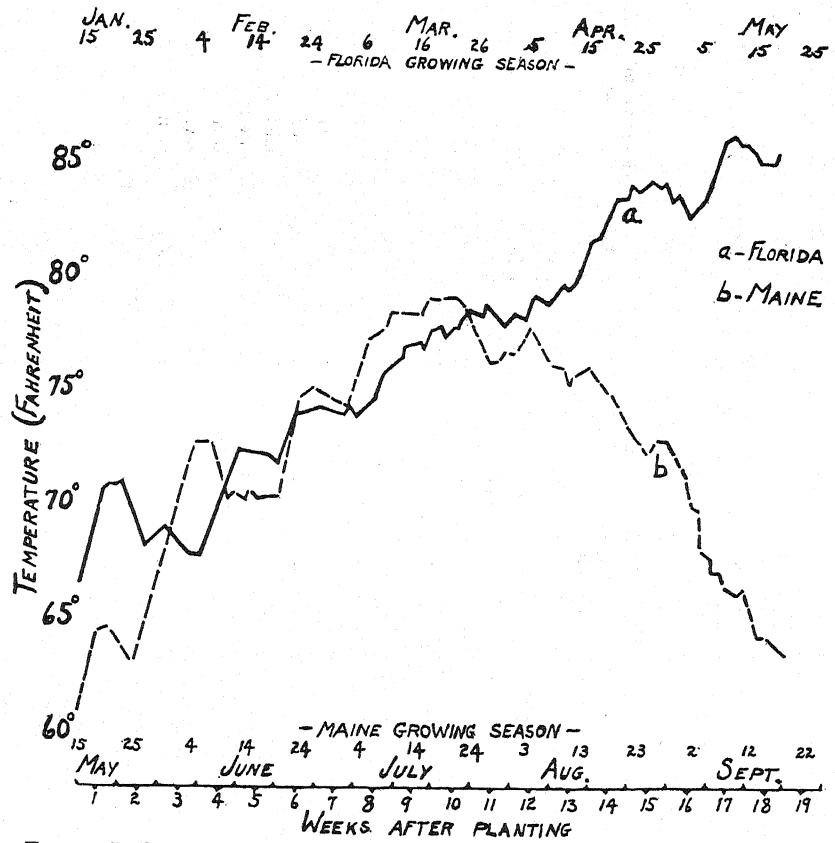


FIG. 1. Daily average maximal temperatures during the ten-year periods 1917–1926 and 1916–1926[1] for the potato-growing seasons at Federal Point, Florida, and Aroostook Farm, Presque Isle, Maine, respectively.

sets of data by plotting a daily moving average of the observations for every 7 successive days. The graphic representation of the normal temperatures thus obtained is shown in figures 1–3. Precipitation records are also given in tables 2–3.

The usual planting dates for most of the acreage in the Hastings and Aroostook County sections average about January 15 and May 15, respectively. It is noteworthy that the Florida temperatures for about the first 3 weeks after planting are higher than those in Maine during the corresponding period (Figs. 1–3). During the fourth week they are lower and the fifth and sixth week they may be slightly higher. From then on the Florida temperatures continue slightly lower until about the middle of the

[1] Data for 1918 missing.

TABLE 2.—*Late blight in relation to the average monthly temperatures and total monthly rainfall from 1914 to 1928 during the potato-growing season at Federal Point (in the Hastings area), Florida*

Year	January		February		March		April		May		Severity of late blight
	Average temp.	Rainfall inches	Average temp.	Rainfall inches	Average temp.	Rainfall inches	Average temp.	Rainfall inches	Average temp.	Rainfall inches	
1914	57	5.10	69	4.00	60	1.69	71	0.94	75	2.56	
1915	58	2.87	56	4.09	57	3.39	68	1.19	79	7.49	
1916	65	0.59	60	0.78	61	0.69	69	0.88	77	3.82	
1917	65	0.96	59	1.59	68	5.91	71	1.68	75	1.03	
1918	53	3.23	66	0.03	70	2.25	69	7.77	75	2.12	
1919	58	1.74	60	4.13	66	8.34	69	2.50	76	4.11	
1920	59	2.87	56	13.52	62	1.31	71	3.79	76	8.01	
1921	60	1.63	61	1.12	70	0.18	70	1.32	74	6.97	
1922	56	3.07	64	4.66	67	1.34	73	1.83	77	6.62	severe
1923	60	1.44	61	1.64	67	2.05	72	1.21	73	10.50	medium
1924	58	3.43	57	2.80	59	11.01	70	2.18	75	2.99	medium
1925	61	3.31	62	1.48	65	2.15	63	0.31	75	2.59	severe
1926	56	3.57	59	1.60	61	3.99	67	.....	74	0.85	severe
1927	58	0.25	66	3.81	65	1.57	72	1.04	77	1.66	severe
1928	55	0.92	61	1.64	66	2.39	68	8.38	73	4.22	none
Average	58.6	2.33	61.1	3.13	64.3	3.22	69.5	2.50	75.4	4.37	

season when the condition is reversed, and the cooler weather persists in the North and the higher temperatures in the South. With such a similarity in temperature from the third to the tenth week, little difference in the severity of certain diseases should be expected, if temperature is the primary governing factor. The actual conditions which have been observed in connection with the most common diseases are discussed below.

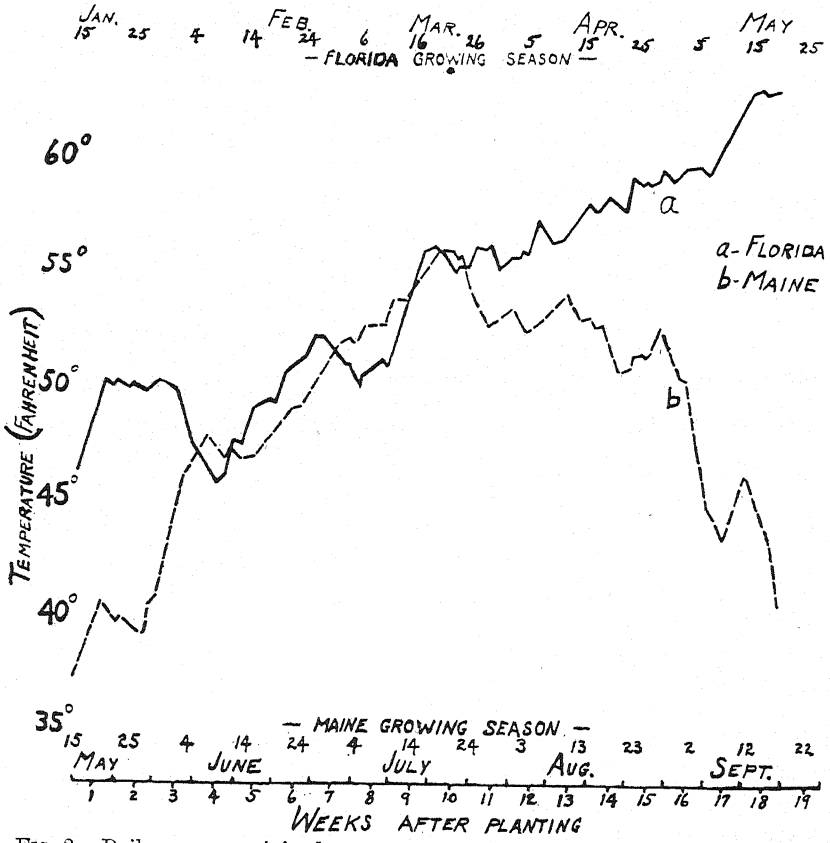


FIG. 2. Daily average minimal temperatures during the ten-year periods 1917–1926 and 1916–1926[1] for the potato-growing seasons at Federal Point, Florida, and Aroostook Farm, Presque Isle, Maine, respectively.

FUNGOUS DISEASES

Late blight (*Phytophthora infestans* (*Mont.*) *de Bary*). The symptoms of this disease, under average conditions, are identical in both sections. By comparing those fields where proper control measures are not practiced one finds, occasionally, slightly greater destruction of foliage in Florida than in

[1] Data for 1918 missing.

TABLE 3.—*Late blight in relation to the average monthly temperatures and total monthly rainfall from 1913 to 1928 during the potato-growing season at Presque Isle, Maine*

Year	May		June		July		August		September		Severity of late blight[1]
	Average temp.	Rainfall inches	Average temp.	Rainfall inches	Average temp.	Rainfall inches	Average temp.	Rainfall inches	Average temp.	Rainfall inches	
1913	49	3.53	61	1.20	69	5.18	61	3.01	54	2.01	medium
1914	53	2.74	56	4.80	64	2.23	60	2.35	56	2.10	do
1915	51	4.05	61	1.95	64	3.40	62	3.50	56	3.25	severe
1916	51	3.45	60	2.17	68	3.68	72	1.70	59	4.05	do
1917	43	3.90	60	7.67	68	2.56	67	5.32	53	1.41	do
1918	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
1919	51	3.32	63	0.80	.....	3.80	61	1.75	53	4.56	10 per cent loss
1920	51	0.91	60	6.08	64	4.28	67	3.62	56	5.21	20 per cent do
1921	53	1.63	59	1.58	72	2.49	61	5.43	56	3.15	0.5 per cent do
1922	52	1.55	63	11.10	65	1.50	62	3.88	55	1.05	5 per cent do
1923	48	1.58	59	0.82	62	3.97	59	2.32	54	.....	0
1924	47	3.03	58	0.76	67	2.09	.....	.....	52	3.34	severe
1925	47	2.32	61	3.21	63	2.45	65	3.09	52	4.18	5 per cent loss
1926	46	1.86	58	1.84	67	2.10	61	3.71	52	3.30	7 per cent do
1927	47	2.08	53	2.32	66	2.94	61	5.25	55	1.36	25 per cent do
1928	50	2.59	56	3.06	66	4.62	65	3.30	52	4.80	severe
Average	49	2.57	59	3.29	66	3.15	63	3.45	54	3.13	

[1] 1913–1918 from Folsom (5).
1919–1926 from *Plant Disease Reporter*.

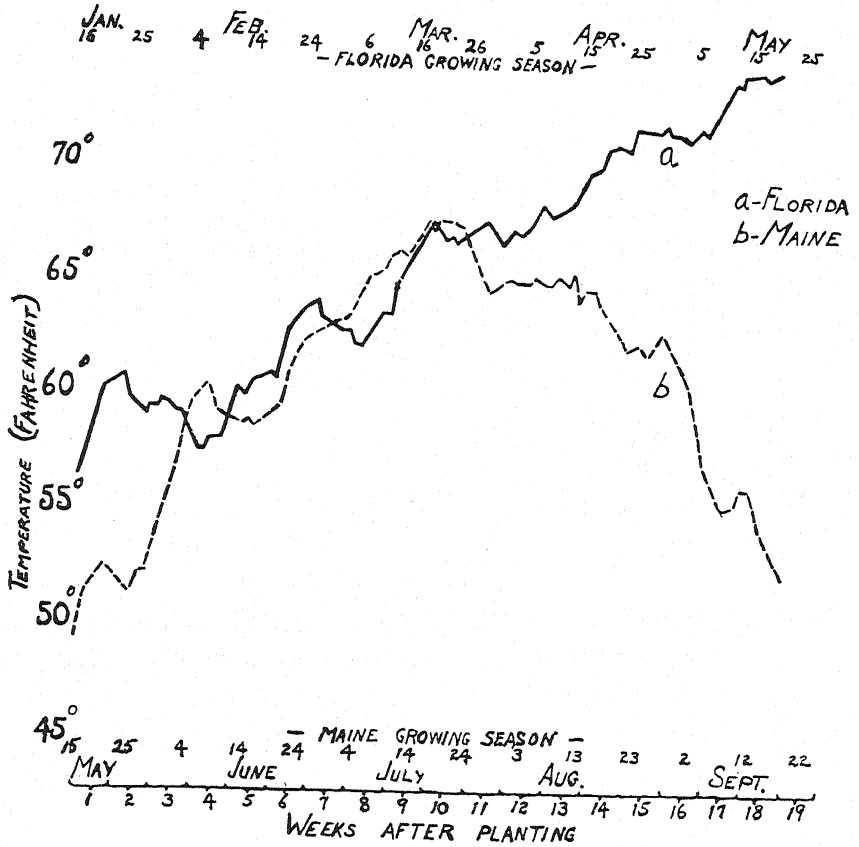


FIG. 3. Daily average mean temperatures during the ten-year periods 1917–1926 and 1916–1926[1] for the potato-growing seasons at Federal Point, Florida, and Aroostook Farm, Presque Isle, Maine, respectively.

Maine, although this is by no means the rule. Much tuber rot may be observed during certain seasons in Maine, but the amount of "mahogany rot" in Florida at time of harvesting is almost negligible except in a few instances of very early (Apr. 1–10) harvesting, where trouble may develop in transit to market.

By applying the known facts to existing conditions it is possible to explain, in a general way, these blight attacks in both sections. Melhus (12) clearly established that the optimal temperature for both the production of motile swarmspores (indirect germination) and infection is from 10 to 13° C. (50–56° F.), and the maximal is about 25° C. (77° F.). He demonstrated also that the optimal temperature for germ-tube or direct germina-

[1] Data for 1918 missing.

tion (*i.e.*, without liberating zoöspores) is about the same as the maximal for indirect germination. Direct germination was observed in his experimental studies, but not in nature, as he definitely states,

> "Indirect germination takes place in the morning dew and rain on potato foliage under field conditions. Direct germination was not observed to occur in the open on the foliage."

Melhus further ascertained that the optimal temperature for mycelial development is approximately 24° C. (75° F.), but that such development, though slower, is possible at lower temperatures.

Folsom, basing his opinion on the results of practical experience (4, p. 152) and on somewhat meager yearly reports of other investigators covering 12 years (5), concluded that the "cool weather" of Aroostook County checked late blight.

By examining the temperature curves (Fig. 1), we observe that the maximal temperatures in Maine throughout July, August, and early September are 21° C. (70° F.) and much of the time above 24° C. (75° F.). Also, it may be observed (Fig. 2) that the minimal temperatures in Maine remain above 10° C. (50° F.) from the middle of July until after the middle of August. Therefore, it appears doubtful if the weather of Aroostook

TABLE 4.—*Late blight in relation to frequencies of minimal and maximal temperatures from 1914 to 1928 during three months of the potato-growing season at Federal Point, Florida*

Year	Number of days in each month with the extremes of temperature indicated						Severity of late blight
	50° F. and below			70° F. and above			
	Feb.	Mar.	Apr.	Feb.	Mar.	Apr.	
1914	16	17	3	15	21	29	
1915	21	25	12	12	15	26	
1916	16	18	6	19	25	29	
1917	15	8	5	17	29	30	
1918	8	3	5	24	29	27	
1919	16	10	2	14	29	30	
1920	21	11	3	10	18	27	
1921	15	4	5	19	30	30	
1922	10	5	0	22	28	30	
1923	9	7	1	19	28	29	medium
1924	12	19	3	13	18	29	do
1925	10	12	6	21	26	29	severe
1926	16	15	11	20	23	30	do
1927	8	13	1	27	29	29	do
1928	17	14	13	16	25	27	none

TABLE 5.—*Late blight in relation to frequencies of minimal and maximal temperatures from 1913 to 1928 during three months of the potato-growing season at Presque Isle, Maine*

Year	Number of days in each month with the extremes of temperature indicated						Severity of late blight[1]
	50° F. and below			70° F. and above			
	July	Aug.	Sept.	July	Aug.	Sept.	
1913	4	16	26	26	27	10	medium
1914	17	22	25	26	22	18	do
1915	19	15	23	29	22	15	severe
1916	8	1	15	28	29	15	do
1917	5	5	29	28	25	11	do
1918	.....	.....	.....	.....	.....	.....	.....
1919	.....[2]	15	27	.....	20	6	10 per cent loss
1920	10	9	18	24	31	10	20 per cent do
1921	6	19	21	31	22	11	0.5 per cent do
1922	12	15	21	26	17	13	5 per cent do
1923	19	19	26	22	16	9	0 per cent do
1924	9	.....	26	26	.....	4	severe
1925	9	8	26	25	26	5	5 per cent loss
1926	10	17	29	25	22	4	7 per cent do
1927	7	15	23	30	25	9	25 per cent do
1928	12	8	26	27	24	6	severe

[1] 1913–1918 from Folsom (5).
1919–1927 from *Plant Disease Reporter*.
1928 from writer's personal observations.
[2] Indicating that no data are available.

County during the actual growing season is ever cool enough to greatly check late blight. In fact, the temperature more nearly approaches that reported by Melhus as the optimal for mycelial development of the fungus. The high percentage of moisture in the Maine fields, both in the form of dew and rain, also is favorable for the development of the fungus. Therefore, if the optimal temperature for development of the disease coincided with that for growth of the mycelium, it seems that little more could be desired to make conditions favorable for development of blight in the Maine fields. On the other hand, in view of the report of Melhus, it does not appear surprising to find epiphytotics of late blight in Maine fields after the middle of August when the minimal temperatures drop to about 10° C. although the maximal temperatures remain above 21° C. (70° F.) and that its failure to develop earlier in the season possibly is due to the higher minimal temperatures. The latter statement is supported by the investigations of Reddick under parallel conditions in New York (19). He demonstrated that, while

sporangial germination is possible either below or above 10° C. (50° F.), a temperature of 15.5° C. (60° F.) is too high for field infection of any appreciable amount. He presents experimental evidence to show that, while 21° C. (70° F.) is most favorable for swarmspore germination and penetration, the inhibiting factor for blight development under his conditions was not the failure of the swarmspores to germinate, but the failure of swarmspore production, as "the temperature of 60° (F.) was not low enough to initiate germination of the sporangia."

From the above conclusions we would expect that with sufficient moisture and high enough maximal temperatures during the day, the more frequently the minimal temperatures hover around 50° F. (10° C.) the more severe will be the epiphytotic. Table 5, based on climatological data for the 15-year period from 1913–1928 for Presque Isle, Maine, and giving the number of days during the growing season that the minimal temperatures were 50° F. or below, indicates that this theory is incorrect. It is observed there that such low temperatures in August are frequent, and yet the reports that blight was of but little consequence are also numerous. In the 15 years for which Presque Isle records are available, from 1 to 22 days in every July and August were found with minimal temperatures of 50° F. or less. There is then no reason to believe that blight was inhibited in Maine because the temperatures were too high. The average maximal temperature curve for Presque Isle (Fig. 1) indicates that ordinarily the temperatures were high enough for the vegetative growth of the blight organism (75° F.). Folsom and Bonde (5), giving average temperatures for 1913 to 1924, however, show that within that period a definite relationship between low temperatures in August and the absence of blight even with abundant moisture existed for but a few years. By comparing the average (mean) August temperatures from 1913 to 1928, with the occurrence of blight (Table 3), such an agreement is found for 1921, 1922, and 1923, where blight is not severe even with abundant moisture. This relationship is particularly apparent for 1921 and 1922 but not so pronounced for 1925 and 1926, where the injury was not exceedingly severe even though the temperatures were more favorable, especially in 1925. However, no such humidity-temperature relation is found for 1927. It is especially noteworthy that the August data for average temperatures and total rainfall for 1921 and 1927 are practically identical, and yet the injury was as severe in 1927 as it has been for many years, while in 1921 blight was almost negligible. It is true that in 15 years for which data are available 4 years (1916, 1917, 1920, and 1925) had both an average August temperature of over 65° F. (18° C.) and a severe epiphytotic of late blight (loss of 5 per cent or over), but it is also true that at least 6 of the 15 years had average August temperatures below 65° F. (mostly about 60–

TABLE 6.—*Comparative results obtained with samples of the same lots of healthy and Verticillium wilt-infected potatoes planted in Maine and Florida*

Seed stock	Aroostook Farm, Presque Isle, Maine: No. of repl.	Stand	Wilt Per cent	Yield per acre (bushels): Primes	Seconds	Culls	Hastings Laboratory, Hastings, Florida: No. of repl.	Stand	Wilt Per cent	Yield per acre (bushels): Primes	Seconds	Culls
				1926						*1926*		
Healthy	6	99	0.0	327.2	13.1	4.4	6	100	0.0	No yield records taken		
Wilt-infected	5	97	25.5	258.9	6.8	6.8	6	100	3.0[1]			
Decrease												
bushels				68.3	6.3	− 2.4						
per cent				20.9	48.1	−54.6						
				1927						*1927*		
Healthy	6	93.5	1.5	404.6	17.7	.....	6	99	0.0	No yield records taken		
Wilt-infected	6	95.5	30.0	346.5	28.0	.....	6	98	0.0			
Decrease												
bushels				58.1	−11.3							
per cent				14.3	64.8							
				1928						*1928*		
	The experiment not conducted at Presque Isle this season						One bushel planted from wilt-infected seed stock					
							Two per cent of the plants indicated a slight vascular discoloration of the stem					
							No stem end browning in any of the tubers					

[1] Symptoms very slight.

61° F.) (15–16° C.), with a blight loss of 5 per cent or over. Again, since there are but 2 or 3 years where there is an actual relation between low average August temperature (approximately 60° F.) and no appreciable amount of blight, it is also true that during these 15 years, 1917 and 1920 are the only outstanding seasons where comparatively high August temperatures and precipitation records are associated with a severe blight attack, with the possible exception of 1928. It is possible that were records available for 100 years for Presque Isle, the conclusion that cool weather checks late blight could be reached, but at present such a conclusion seems unwarranted.

The fact is well established that the causal organism thrives under certain conditions of moisture, but, here, again, upon examining the climatological data (Table 3), no definite relation can be found and the indications are that in practically every year in Aroostook County there is sufficient moisture for the development of the fungus. The relation of high moisture and severe blight seems definite for 4 years where, in 1920 and 1928, the August rainfall was 3.3 inches or more and, in 1917 and 1927, over 5.0 inches. However, it is also to be noted that the rainfall for August, 1927, was the same as for August, 1921, and the losses from blight during those two years were 25 per cent, and ½ per cent, respectively, in spite of the fact that the mean temperatures for both years were identical (60.7 and 60.9° F.). No explanation for this difference has been advanced.

Attempts to show a relation between cool and wet, cool and dry, or warm and wet, and warm and dry weather and the occurrence of blight have not been very successful, as severe losses have been tabulated for Maine for all of these conditions, even in the few years for which records are available.

Upon returning now to the temperature data (Fig. 2), we observe that the minimal temperatures for Florida during the second month after planting are about the same as those in Maine during the similar portion of the growing season. During the third month after planting, however, the temperatures are more nearly the optimal for sporangial germination in Florida than in Maine. Blight is usually first observed in the Hastings belt any time after the first of March or even earlier, but the attack may be severe until late April, especially if the season is late. A study of the minimal temperatures (Fig. 2) will indicate why this is possible. Table 4, which gives the status of the minimal and maximal temperatures for the Florida area, indicates that the temperatures are both low enough for the initiation of an attack and high enough for mycelial development. A definite relation between climatic conditions and the severity of blight attacks cannot be established for this section because no definite records of epiphytotics are available except for the last few years.

Martin (11), in summarizing the work of others, points out clearly that the different investigators are not in accord as to the climatic conditions which are most favorable for the development of this disease. In presenting his own data, however, he indicates that definite correlations between temperatures and moistures and outbreaks of late blight are possible if the observations of many years are available. It is evident that the Florida and Maine data here presented are not sufficient and of long enough standing to answer definitely the questions which are constantly being presented. As Reddick (19) suggests,

> "It is unlikely that the existing records of weather are sufficiently detailed to give the minutiae necessary for the complete analysis of the situation, but rainfall and temperature records alone would probably give a more complete explanation of the conditions affecting the incidence of the disease than now exists."

These temperature records indicate the similarity between Aroostook County and Hastings conditions but do not always demonstrate to the investigator or to the grower what he may expect under the various existing climatic conditions. Only the records of many score more years will warrant more accurate predictions if such are to be made from past field experiences.

Early blight (*Alternaria solani* (*E. & M.*) *J. & G.*). Early blight causes about the same amount of damage in Florida as it does in Maine. Usually in both sections this trouble does not appear until the plants are approaching maturity. Occasionally the disease may appear earlier, but only in isolated instances where conditions have been particularly favorable for its development. In 1928 the disease was much more severe in Maine than during the 4 preceding years. This was also true for Florida, where the actual losses from this trouble alone were comparatively small. By comparing these facts with experimental evidence it is at once apparent that the field observations as made are about what could be expected. Rands (18) demonstrated definitely that the optimal temperature for both spore production and mycelial growth is about 26–28° C. (79–83° F.) but that growth is possible down to 1–3° C. (35° F.), and up to 37–45° C. (99–113° F.) or over. He further determined that, while heavy dews were an aid, abundant spore production took place only when such dews were supplemented by rainfall. Whetzel (28) likewise reports a serious epiphytotic of early blight in Bermuda following a season of warm, wet weather.

Examination of the temperature records (Figs. 1–3) shows that, with such a wide range between the maximal and minimal limits, there is no reason why this trouble should not develop in either section. Precipitation records (Tables 2–3) also indicate sufficient moisture in both sections, with a somewhat heavier rainfall at the end of the season in the South than at the end of the season in the North. It is reasonable to assume that the

greater precipitation abetted by the higher temperature toward maturity may be responsible for the slightly greater degree of severity of this disease in Florida, but the total percentage loss in either section as compared with that caused by late blight is small.

Rhizoctonosis (*Corticium vagum B. & C.*). Rhizoctonosis, perhaps better known as "black scurf" or the "little potato disease", much discussed as it has been, still presents a problem which is far from being solved. Richards' (20, 21) results demonstrated that this fungus may be parasitic through a range of from 9 to 30° C. (48–86° F.). He observed the greatest number of stem lesions at 12–21° C. (54–70° F.), with about 18° C. (64° F.) as the temperature where the most serious injury occurred.

Occasionally a small amount of injury may be observed in the very early (December) planted fields, but the percentage of "stem cankering" observed in the Florida territory was comparatively small, and there was seldom any killing of the tips of the stems, while the total number of hills affected with rhizoctonosis later in the season was always exceedingly small. In Maine, however, a rather high percentage of stem lesions and often considerable tip killing are observed and the number of plants manifesting typical rhizoctonosis symptoms later in the season is much larger than in Florida. The total loss in either section is not anywhere near 50 per cent, as Richards reports for Utah (22). In Maine the stem lesions frequently involve the entire stem below the surface of the ground and persist during the entire season. In the Florida potato belt the lesions seldom attain a length greater than from a quarter to a half inch and have not been observed to increase in size after the plants have become definitely established.

A comparison of the temperature curves (Figs. 1–3) indicates that in both sections the fungus should thrive during the 4- to 6-week period immediately after planting. With the knowledge that the actual damage occurs chiefly in the Maine fields, even though the temperatures are very similar in the early part of the growing season, it is evident that more extensive observations and experiments are necessary in both sections before a satisfactory explanation of the differences can be given. Soil-temperature data are not available. It is possible that these would not be so different in the two sections, and in that event an adequate explanation for this difference of behavior in the two sections must be sought elsewhere. One possibility is that the seed pieces in Florida are planted about 3 inches deep, on ridges, while in Maine the depth is almost twice as great and planting is practically on the level. Also, the soil is very sandy in the former section, and the surface layer, at the point where the fungus ordinarily is most destructive, may dry out much more quickly than in Maine where the soil is heavier and where planting is not on ridges.

Scab (*Actinomyces scabies* (*Thax.*) *Güssow*). Scab is considered of no commercial importance in the Florida potato section, and neither is it considered very destructive in Maine, although it is much more important in the latter section. The reason which Gillespie and Hurst (6) advance for the mild attacks in Maine is, ". . . an excellent correlation between the hydrogen-ion concentration (of soils) and occurrence of common potato scab." Their examination of a large number of soils from northern Maine revealed that those with a pH value of 5.2 or less rarely produced scabby potatoes. They found a difference in hydrogen-ion exponent between the Caribou and the Washburn loam, with a pH of 4.8 in the former and a much higher value in the latter, and the former producing scab-free potatoes, while those from the Washburn type were usually scabby.

Several score hydrogen-ion determinations were made on soils from different sections of the Hastings belt and they varied consistently in pH from 5.4 to 5.8. These figures are still within the lower limit of tolerance (pH 5.2) for scab development, but may be low enough for a certain amount of deterring effect. It is usually considered by the growers that the Hastings soils are too acid for the development of this disease. Whether or not other factors are involved remains to be determined. Millard and Burr (13) suggest the possibility of the existence of different species of the scab organism in soils of different reaction and texture, and ". . . under certain extreme conditions either of climate or soil, tubers are parasitized by one species or one cognate group of species to the exclusion of the others." Millard and Taylor (14) and Sanford (23) demonstrate the possibility of a certain balance between various parasitic and saprophytic fungi, and possibly bacteria, and that the particular rapid growth of the nonparasitic organisms, induced by the turning under of green cover crops, retards or inhibits the development of the scab pathogene, possibly through a lack of the proper nutrient materials, which results in the starvation of the parasitic type. Millard and Taylor suggest this possibility for soils with a comparatively high pH, while in other soils a high hydrogen-ion concentration may itself be the controlling factor. In the Hastings section a heavy growth of cowpeas and various native grasses is turned under annually, but the actual value of such green manuring, from the standpoint of inhibiting scab, is unknown. In Maine a similar procedure is followed, in part, where clover is turned under in the fall and potatoes are planted the following spring. It is reasonable to assume that such other hidden factors are involved, since the outstanding environmental conditions appear favorable for the growth of the causal organism. For example, Jones, McKinney and Fellows (8) have demonstrated that comparatively high temperatures are favorable for scab, and our temperature records (Table 1) indicate that, in general, the mild-

ness of this trouble is not due to unfavorable climatic conditions. Further intensive investigations are necessary in both sections to explain these differences.

Verticillium wilt (*Verticillium albo-atrum Reinke & Berth.*). In Maine a condition exists which is comparable with similar conditions reported from such other Northern States, particularly Oregon, where Verticillium wilt may at times be extremely serious. This trouble, which supposedly causes no decay of the tubers but causes a vascular discoloration of the stem and tubers and a great reduction in yield, is considered as existing only in the northern areas where cooler temperatures prevail. Chupp (2), however, mentions that "in New York where the summer temperatures are relatively low there are only occasional farms where the latter fungus is destructive," and thinks that the distribution is regional.

It has been well demonstrated that this fungus is disseminated through the seed potatoes and that it remains in the soil for many years. This being a fact, it is reasonable to expect that, other conditions being equal, the Florida soils should contain sufficient inoculum, considering the length of time that seed stock has been imported and planted from a region where this trouble is extant. Verticillium wilt has been seldom observed in Florida, even though the seed stock frequently comes from Maine fields where rather high percentages were present.

In order to gain more definite information regarding this trouble 2- to 3-bushel samples of tubers were selected during several consecutive seasons in Maine[3] from hills which had been killed by this organism. The tubers were carefully examined and each one was rejected which did not have the typical stem-end browning.

These samples were divided into two lots, and during the following season one lot was planted in Florida and the other in Maine. In each plot the sample was compared directly in alternate rows in several replications with tubers taken from wilt-free hills. The percentage of hills exhibiting symptoms of the disease was determined each year. These figures are given in table 6. Practically no wilt was observed on these plantings in Florida at any time during the season, while comparatively high percentages of wilt occurred in the same stock (Table 6) when planted in Maine. Comparative yield data were obtained in Maine but not in Florida because in the latter section the plants both from clean and diseased tubers showed no differences and apparently all were healthy. Why this disease is severe in one of these sections and not in the other is unexplained. Edson and Shapovalov (3) when comparing in culture two strains of *V. albo-atrum* discovered that the one from Maine shows a much better adaptation to

[3] This experiment was conducted in cooperation with Doctor E. S. Schultz, Senior Pathologist, Bureau of Plant Industry, U. S. Department of Agriculture.

lower temperatures while the strain from West Virginia exhibits a better adaptation to higher temperatures, the former giving no growth and the latter giving fair growth at 30° C. (86° F.). Reasoning from the results of these investigators, the conclusion would be reached that, since the temperatures are so nearly alike in both sections for part of the growing season and since the maximal temperature for the growth of this fungus is at no time approached until the latter part of the season, Florida fields should suffer severe losses through Verticillium wilt. It is possible that the increase in temperature in this southern area may aid in retarding the development of this fungus, but it is reasonable to believe that in some sections of the potato area and in some seasons at least mild symptoms should be observed. Such conclusions have not been confirmed by the observations of the writer during the last 6 years. Again, soil temperatures are not available for both sections, but the air temperatures given above serve as an index of what the soil temperatures may be, and it is possible that other factors are also involved.

BACTERIAL DISEASES

Blackleg (*Bacillus atrosepticus van Hall*). The blackleg situation presents another interesting problem. This disease was much discussed and investigated a decade or so ago. Morse (15, 16, 17), and others, later, found that consistent treatment and careful selection of the seed stock gave good commercial control, and, until several years ago, this disease was considered as becoming less important. Two years ago, however, it gained a fresh momentum when, in Maine and other northern potato-producing States, very high percentages of this trouble occurred. In Maine high percentages were found also in fields planted from seed stock in which, the preceding year, no trace of this disease had been observed. Several of the best growers in Aroostook, who had carefully hand picked their seed potatoes from tuber unit material and had treated them with the highly recommended hot-formaldehyde method, were not granted any immunity but suffered fully as great injury as others who followed the ordinary routine methods of planting average seed potatoes.

Various investigators at once began to theorize and search for satisfactory explanations, only to be baffled a second year with a recurrence of similar high percentages. In the Maine fields diseased stalks were observed frequently which showed typical symptoms of this disease on parts above ground, while on the remainder of the stem and on the seed piece there was no decay or discoloration of any sort. This suggests the possibility of insect transmission in the Maine fields, as was found to be the case in Minnesota by Leach (10). The problem is by no means solved, but the fact that the seed-corn maggot (*Hylemyia cilicrura* Bond.) can be responsible has

been confirmed recently by Bonde (1). Whether other insects or other factors are concerned has not been demonstrated.

During these several years of high percentages of blackleg infection in Maine the Florida fields were carefully observed, but nowhere has the amount increased perceptibly over previous years. Only a slight trace of this disease has been evident in Florida for the last 6 years.

In addition to field observations for the past several seasons, tubers were carefully selected from blackleg hills of the Spaulding Rose No. 4 variety in Maine fields and were planted in Florida. Upon arrival in Florida the tubers always showed much rot because only those were selected in Maine which showed unmistakable symptoms of the disease. Precautions were taken to plant only those seed pieces which were partly decayed or which had been covered with the decayed material from other tubers. Frequently, the potatoes were split lengthwise to insure the presence of a decayed portion on each seed piece. In 1928 but 3 hills manifested the disease in a total of 39 tuber unit samples of 4 hills each, and each one of these diseased hills was in a different unit. In 1927 similar results were obtained and not over 3 to 4 per cent blackleg hills were observed in the rows planted with such diseased material. Such failure to produce the disease by planting diseased tubers has been reported by other investigators and has not been explained satisfactorily. Again, the possibility of insect dissemination or absence of such spread is suggested. The seed-corn maggot (*Pegomyia fusciceps* Zett.) was reported from Florida in 1905 (26).

Bacterial wilt (*Bact. solanacearum EFS.*). Bacterial wilt, bacterial blight, and southern brown rot are common names of this disease. According to Smith (27), who isolated the causal organism in 1896, the disease occurs in the United States from Maryland and New Jersey south to Cuba and Porto Rico. The writer has never observed the disease in Maine, and as far as can be ascertained it has never been reported from there.

The disease apparently is favored by high temperatures and appears to be more or less regional, similar in that respect to Verticillium wilt as described by Chupp (2). It is found regularly in some fields year after year, while in other near-by fields only traces have been observed from time to time. The growers have learned from practical experience that early planting and, consequently, early harvesting provide a partial escape from great losses. It is assumed that slightly lower temperature is the controlling factor in these instances. It is not uncommon to observe symptoms of this disease in newly cleared land in Florida and on ground on which solanaceous plants have not been observed. Much experimental work has never been reported, and the total percentage losses, while not extreme, are still large enough to warrant careful investigational work.

Virus diseases (causes not determined). The losses caused by the various degeneration diseases are about as large in the potato fields of Florida as in the Spaulding Rose No. 4 variety in Maine, the percentages being comparatively low in both States. It is to be remembered that the Spaulding Rose No. 4 is about the only variety grown in the Florida potato belt. Since this particular variety apparently is not so susceptible to mosaic as either Bliss Triumph or Green Mountain (7), commercially, the Hastings area has smaller losses through these troubles than Aroostook County. There the relative acreage of other varieties is much greater than that of the Spaulding Rose No. 4. Virus diseases, as they have been observed in both potato sections, will be discussed in a later paper.

SUMMARY

Detailed climatological data, consisting of daily maximal, minimal, and mean temperatures, based on 10 years observations in both the Hastings, Florida, and the Aroostook County, Maine, sections, together with monthly averages of temperature and rainfall, are given and the relations of these facts compared with the occurrence of such diseases as late blight, early blight, rhizoctonosis, scab, and blackleg, which are common to both sections, are pointed out.

Verticillium wilt is shown to be very severe in Maine during certain seasons and negligible in Florida, while bacterial blight is prevalent in Florida and unknown in Maine.

The percentage of virus disease is about the same in the Florida potato section, where the Spaulding Rose No. 4 is the only variety grown, as in the Spaulding fields of Aroostook County.

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STUDIES UPON A BACTERIOPHAGE SPECIFIC FOR PSEUDOMONAS TUMEFACIENS

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Although bacteriophagy in its relation to human and animal pathogenes has been the subject of intensive investigation, little is known as to the occurrence of such lytic principles affecting phytobacteria. In the light of previous work by d'Herelle (8), Cruz (4), Gratia (6), Hauduroy (7), and others dealing with human and animal pathogenes, such considerations in phytopathology as the cause of loss of virulence in bacterial culture, the inactivation or death of bacteria in soil or overgrowths, the occurrence of nonvirulent strains of pathogenes within the host may well be investigated as to their relation to bacteriophagy.

Probably Mallman and Hemstreet (13) were the first to isolate an inhibitive agent from plant material. From a decaying cabbage affected with a fluorescent soft-rot bacterium, they obtained a lytic agent active against the causal rot organism as well as *Bacillus carotovorus* Jones. They also showed that at dilutions of 10^{-17} the bacteriophage would inhibit growth of the soft-rot organism in broth cultures.

At about the same time Gerretsen and Sack at Groningen and Gryns and Söhngen at Wageningen worked independently upon the bacteriophage of the legume nodule organism, *B. radicicola* Beij. Later these investigators (5), in collaboration, published the results of their work on the isolation of a bacteriophage from the nodules, roots, and stems of bean, clover, lupine, and Seradella plants. These investigators, apparently, were the first to demonstrate strain specificity of a bacteriophage from plants. The lytic principle isolated from bean was active against the strain of nodule bacteria from this plant and clover. The bacteriophage from clover nodules affected the organism from clover, but not from lupine nodules. They also isolated a bacteriophage from a culture of the Seradella nodule organism sent them from another laboratory. However, this lytic principle was not active against their own isolation of *B. radicicola* from the same host. Hitchner (10) also obtained a bacteriophage from the nodules of red clover active only against the strain of *B. radicicola* from clover.

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Anderson (1), working with *Ps. pruni* E. F. S., isolated a bacteriophage from the soil beneath a peach tree previously infected with the leaf-spot disease, but did not obtain a lytic agent from old infected leaves, although only one trial was made. The addition of the bacteriophage at high dilutions to agar-plate colonies produced typical plaques, although secondary cultures appeared in these areas after several days.

Coons and Kotila (3) isolated a polyvalent bacteriophage from a carrot rotted by *B. carotovorus*. In this case the lytic principle was active against *B. atrosepticus* van Hall and *Ps. tumefaciens* Smith and Town., as well as the soft-rot organism. After standing five and one-half months the lytic filtrate (ninth passage), although still effective against all the organisms tested, was not capable of causing clearing except in cultures of *B. carotovorus*.

Israilsky (11) later obtained from a crown gall on sugar beet a bacteriophage effective against *Ps. tumefaciens*. From the same gall he obtained two cultures (out of 11 isolations) which were resistant to the lytic principle. Similar resistant strains of the crown-gall organism were isolated from broth cultures to which the bacteriophage had been added in dilutions of 10^{-10} and 10^{-11}.[2] These strains also remained resistant at higher concentrations of the bacteriophage. He was unable, however, to isolate the lytic principle from a pure culture of *Ps. tumefaciens*.

Brown and Quirk (2) recently reported the isolation of a bacteriophage from tumors on *Ricinus communis* L. and sugar beets. In certain cases filtrates from the Ricinus tumors inhibited growth for 20 days when added to young cultures of the crown-gall pathogene. Complete lysis was not obtained in any of the treated cultures, although bizarre forms of the organism were demonstrated. When cultures of *Ps. tumefaciens* treated with high dilutions of the filtrates from tumors and rotted carrots were inoculated into Ricinus plants, galls were induced more rapidly than in the controls and were larger. The addition of such filtrates to young cultures of *Ps. tumefaciens* also stimulated growth. These investigators were unable to demonstrate the presence of an infectious filterable form of the crown-gall pathogene.

The writers (14) previously reported the results of experiments showing the lytic action of two bacteriophages isolated respectively from a crown gall on sugar beet and from a pure culture of *Ps. tumefaciens* (raspberry isolation). It was shown also that the bacteriophage was specific in its lytic action against certain plant pathogenes and isolations of the crown-gall organism in that it affected only the strain of *Ps. tumefaciens* from which it

[2] As a matter of convenience the negative exponent is used to express dilutions. Thus the expression 10^{-2} denotes a distribution of 1–100, 10^{-6} a dilution of 1–1,000,000, etc.

was isolated. These studies have been extended and the results are given in more detail in the present paper.

METHODS AND MATERIALS

The lytic principles (bacteriophages) were obtained from five sources as follows: I, a culture of *Ps. tumefaciens* in unsterilized soil no longer infectious; II, a crown gall on *Rumex crispus* L. produced by artificial inoculation with the Rumex strain of *Ps. tumefaciens*; III, a sugar-beet gall induced by artificial infection with the raspberry isolation of *Ps. tumefaciens*; IV, a culture of *Ps. tumefaciens* in sterilized soil no longer infectious; and V, a virulent culture of *Ps. tumefaciens* isolated from a raspberry gall in 1923. In this connection it should be noted that the soil cultures were originally inoculated January 12, 1926, with the raspberry isolation of the crown-gall organism. The sugar-beet gall was induced by inoculation with a subculture of the organism from which lytic principle V was obtained. Thus bacteriophages I, III, and IV were obtained from inoculation with subcultures of the same isolation of *Ps. tumefaciens.*

In obtaining lytic principles I and IV, 10 grams of the soil were placed in 30 cc. of neutral peptone-dextrose broth and incubated at room temperature for thirty hours. The cultures were then filtered through paper and the filtrate passed through a Berkefield W filter. Four days later 20 cc. of a 24-hour broth culture of *Ps. tumefaciens* was added to the clear filtrate and after standing three days was filtered as before. This procedure of adding cultures of the organisms and filtering was repeated at intervals of four to seven days or longer.

Lytic principles II and III were obtained by filtration from cultures made by adding 20 grams of the finely ground galls from sugar beet and *R. crispus* to 50 cc. of neutral peptone-dextrose broth. The cultures were incubated 24 hours at room temperature, filtered through filter paper, and then through Berkefeld filters. Subsequent additions of young broth cultures of *Ps. tumefaciens* to the filtrates and filtrations were made as given above. Lytic principle V was obtained from a seven-day-old neutral-broth culture of *Ps. tumefaciens* isolated from a raspberry gall in 1923 and carried as a stock culture for inoculation studies. The method employed was a series of filtrations through Berkefeld filters with additions at irregular intervals of 10 cc. of a 24-hour broth culture to the sterile filtrate. At different times during the progress of these successive additions and filtrations, tests were made of the presence of a lytic principle in the filtrates.

EARLY STUDIES OF THE BACTERIOPHAGE

After four successive filtrations one cc. of the filtrates I to V, inclusive, was added to 9 cc. of a 24-hour-old broth culture of the raspberry strain (R)

of *Ps. tumefaciens*. This procedure was repeated, using different dilutions of the filtrate so as to make the final dilution of the lytic principle 10^{-2} to 10^{-6}, inclusive. Readings taken at the end of seven days showed growth in all the dilutions of lytic filtrates I, II, and IV but in none of the dilutions of filtrates III and V.

After seven filtrations, again one cc. of each of the filtrates I to V, inclusive, was added to sterile broth, making dilutions of 10^{-2} to 10^{-6}, inclusive. To each of the diluted filtrates was added one cc. of a 48-hour broth culture of *Ps. tumefaciens* and records taken at the end of seven days. The results of this trial are given in table 1.

TABLE 1.—*The effect of lytic filtrates in different dilutions upon the growth of Pseudomonas tumefaciens*[a]

Lytic filtrate	Dilutions of filtrate and growth reactions					
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Check
I	4	4	4	4	4	4
II	4	4	4	4	4	4
III	0	0	0	0	0	4
IV	0	0	0	0	0	4
V	0	0	0	0	0	4

[a] Growth is indicated as follows:
0 = No growth, broth clear.
1 = Very slight growth, very faint turbidity of broth.
2 = Slight growth, faint turbidity of broth.
3 = Moderate growth, slight turbidity of broth.
4 = Abundant growth with pellicle formation.

Although the filtrates I and II showed growth at dilutions of 10^{-2} to 10^{-6}, inclusive, there was a certain amount of the lytic agent present in the undiluted stock filtrate. The addition of 10 cc. of a 24-hour culture of *Ps. tumefaciens* to 30 cc. of the filtrates above mentioned failed to show clouding of the culture until five days had elapsed and then only a slight turbidity was evident.

To test further the lytic action of the five stock filtrates, inoculations were made into tomato plants. For this purpose 10 cc. of a culture of *Ps. tumefaciens* was added to 30 cc. of the stock filtrate and allowed to stand two days. At this time there was no visible growth in the filtrates. Inoculations were made by needle prick into the tips of succulent young tomato plants. No galls developed from the filtrate inoculations while the checks inoculated with *Ps. tumefaciens* showed typical galls twenty-four days later.

EFFECT ON PSEUDOMONAS TUMEFACIENS OF CONTACT WITH LYTIC FILTRATES OF DIFFERENT DILUTIONS AND FOR DIFFERENT LENGTHS OF TIME

One cc. of a 24-hour broth culture of *Ps. tumefaciens* was added to one, two, three, four, and five cubic centimeters of the stock lytic principle III. At this time the lytic filtrate had undergone fourteen series of additions and filtrations and had been standing two months since the last series. The above mixtures, after standing for different periods of time ranging from three to 60 hours, were used in inoculations of young tomato plants.

Two plants were inoculated by needle with each mixture, ten punctures being made in each stem. Results of these trials 30 days after inoculation are presented in table 2. Table 2 shows that inoculations with all the mixtures at the end of three hours produced galls, showing that *Ps. tumefaciens* had not been inactivated up to that time. At the end of six hours, however, at a concentration of one part of the suspension of the organism to two parts lytic filtrate, and greater, no galls were produced. At concentrations of one to four and one to five, slight smooth swellings developed at the edge of the punctures, but attempts at reisolation using bile agar failed to recover the pathogenes from these swellings. After nine hours and until the termination of the experiment at 60 hours no galls were produced by inoculation with the mixtures at any of the concentrations. In all cases the lytic filtrate

TABLE 2.—*Results of inoculation into tomato of a mixture of one cc. of a Pseudomonas tumefaciens suspension with different quantities of lytic filtrate III after reacting for various lengths of time*[a]

Contact periods hours	Proportions of suspension to lytic filtrate III					Checks	
	1–1	1–2	1–3	1–4	1–5	*Ps. tumefaciens* 1–0	Lytic filtrate III 1–0
3	2[b]	1	1	1	0	2	0
6	1[b]	0	0	1	0	2	0
9	0	0	0	0	0	2	0
12	0	0	0	0	0	2	0
24	0	0	0	0	0	2	0
48	0	0	0	0	0	2	0
60	0	0	0	0	0	2	0

[a] The results of inoculations are indicated as follows:
0 = No galls formed.
1 = Small swelling, not typical galls.
2 = Typical crown gall.

[b] *Ps. tumefaciens* was reisolated from the gall.

alone and *Ps. tumefaciens* were inoculated into tomato plants as checks. In these trials, the lytic filtrate alone failed to produce galls, and *Ps. tumefaciens* consistently produced typical crown galls.

It is interesting to note in this connection that Israilsky (12) showed that rape plants inoculated with a culture of *Ps. tumefaciens* treated with bacteriophage showed a material reduction in the percentage affected with crown gall compared with those inoculated with untreated cultures. He also soaked rape seed two hours in the lytic filtrate at dilutions of 1 to 10 and 1 to 100, to which *Ps. tumefaciens* had been added. In each case there was a decrease in the number of plants with crown gall after treating the seed with the bacteriophaged cultures as compared with untreated cultures.

Further attempts were made to prevent crown-gall infection by the use of bacteriophage. In this experiment one drop of the undiluted lytic filtrate V was injected into the succulent tips of a series of young tomato plants and the point of injection marked with India ink. These trials were made in triplicate. At intervals of two days, up to two weeks, beginning two hours after the injection, *Ps. tumefaciens* was introduced into the wound made by the hypodermic needle. In no case was crown-gall infection prevented. Why the bacteriophage after two hours in the plant did not inhibit the growth of *Ps. tumefaciens* is not clear. It is possible that the acidity of the sap of the tomato plant may have inactivated the lytic agent since it is well known that the bacteriophage is most active in media with neutral or slightly alkaline reactions.

INCREASE IN POTENCY OF THE LYTIC PRINCIPLE AFTER SUCCESSIVE FILTRATIONS

At varying intervals the potency of the lytic principles was tested by making dilutions in alkaline or neutral peptone-dextrose broth and inoculating the tubes with the crown-gall organism. For this purpose filtrate III after three and seven filtrations was employed. The filtrate was made up to dilutions of 10^{-2} to 10^{-6} by the addition of one cc. of the lytic filtrate to 9 cc. of alkaline broth. To each 10 cc. of the diluted filtrate was added one drop of a 24-hour-broth culture of *Ps. tumefaciens* (original raspberry isolation). The results of these trials are shown in table 3.

While the filtrate after three filtrations did not entirely prevent growth, there was distinct evidence of partial inhibition after seven days. At a dilution of 10^{-2} there was only a very faint turbidity of the culture which became more pronounced as the dilution became greater. However, in none of the dilutions was the culture so turbid as in the untreated check culture of *Ps. tumefaciens*. After seven filtrations, the filtrate at all dilutions remained clear seven days after inoculation with *Ps. tumefaciens*. The writers (14) have already shown that after 17 passages through Berkefeld

TABLE 3.—*Increase in potency of the lytic principle after successive filtrations*[a]

Filtrate III	Dilution of filtrate and growth reaction					
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Check
Third filtration	1–	1	2	2	2	4
Seventh filtration	0	0	0	0	0	4

[a] Growth is indicated as follows:
0 = No growth, broth clear.
1– = Very faint haze but less growth than 1.
1 = Very slight growth, very faint turbidity of broth.
2 = Slight growth, faint turbidity of broth.
3 = Moderate growth, slight turbidity of broth.
4 = Abundant growth with pellicle formation.

filters, lytic filtrates III and V at dilutions 10^{-10} and 10^{-14} caused lysis of 24-hour-old cultures of the crown-gall organism.

Further trials were made of the potency of the lytic filtrates III and V after 19 passages, respectively, through Berkefeld W filters. Each series of trials was repeated three times with the same results. In these trials the dilutions of the lytic filtrates were made by adding 0.5 cc. of the liquid containing the bacteriophage to 5 cc. of neutral peptone-dextrose broth. With the same pipette 0.5 cc. of the first dilution (1–10) was withdrawn and added to the second tube until five dilutions were made. A fresh sterile pipette was then used in making the next five dilutions and so on for the entire series. With this method of making the dilutions it is possible that a certain amount of the lytic principle may have been carried over on the walls of the pipette, although it was emptied at each dilution and thoroughly flamed to steaming before making the next dilution. The reaction of the lytic principles upon *Ps. tumefaciens* at dilutions greater than 10^{-30} is given at intervals of 10 because in all cases the growth of the organism was the same as in the check tubes of untreated broth. There was no growth in the tubes containing dilutions of the filtrate at 10^{-1} and 10^{-19}, hence in the table these data are omitted. The data from these tests are given in table 4.

From this table it is seen that at dilutions of 10^{-1} to 10^{-21} both lytic filtrates inhibited growth of *Ps. tumefaciens* for 120 hours following the addition of the inoculum. After 144 hours turbidity occurred to the extent of a faint haze in lytic principle III at a dilution of 10^{-21}. At 168 hours the turbidity had increased to the extent that the medium was slightly cloudy. At this dilution there was no evidence of growth of *Ps. tumefaciens* in the tubes containing lytic principle V. At dilutions of 10^{-22} very slight

TABLE 4.—*The effect of lytic filtrates V and III at various dilutions upon growth of Pseudomonas tumefaciens*[a]

Dilution of filtrate	Lytic filtrate V							Dilution of filtrate	Lytic filtrate III						
	Growth of culture Hours								Growth of culture Hours						
	24	48	72	96	120	144	168		24	48	72	96	120	144	168
10^{-20}	0	0	0	0	0	0	0	10^{-20}	1–	0	0	0	0	0	0
10^{-21}	1–	0	0	0	0	0	0	10^{-21}	1–	0	0	0	0	1–	1–
10^{-22}	1–	0	1–	1–	1–	1	1	10^{-22}	1–	0	0	1–	1–	1	2
10^{-23}	1–	0	1–	1–	1–	2	3	10^{-23}	1–	0	0	1–	1–	1	3
10^{-24}	1–	0	1–	1–	1–	2	2	10^{-24}	1–	0	0	0	1–	1	3
10^{-25}	1–	0	1–	1–	2	2	3	10^{-25}	1–	0	0	1–	1–	1	3
10^{-26}	1–	0	1–	1–	2	3	4	10^{-26}	1–	0	0	1–	1–	1	3
10^{-27}	1–	2	2	3	4	4	4	10^{-27}	1–	0	1–	1	2	2	3
10^{-28}	1–	2	2	3	4	4	4	10^{-28}	1	0	1–	1	2	3	4
10^{-29}	1–	2	2	3	4	4	4	10^{-29}	1	2	2	2	3	4	4
10^{-30}	1	2	2	3	4	4	4	10^{-30}	1	2	3	2	3	4	4
10^{-40}	1	3	3	3	4	4	4	10^{-40}	1	3	3	3	4	4	4
10^{-50}	1	4	4	4	4	4	4	10^{-50}	1	3	3	3	4	4	4
Check *Ps. tumefaciens*	1	4	4	4	4	4	4	Check *Ps. tumefaciens*	1	4	4	4	4	4	4
Check filtrate V	0	0	0	0	0	0	0	Check filtrate III	0	0	0	0	0	0	0

[a] Growth is indicated as follows:

0 = Clear liquid with few climps of agglutinated bacteria.

1– = Very faint haze but less growth than 1.

1 = Slight turbidity.

2 = Medium turbidity.

3 = Moderately heavy turbidity.

4 = Heavy turbidity and pellicle formation.

turbidity showed in the tubes of both lytic filtrates after 24 haurs. However, the cultures cleared by the end of 48 hours. After 72 hours filtrate V at dilutions of 10^{-22} to 10^{-26} showed a very faint turbidity with a few clumps of the agglutinated bacteria. This condition remained unchanged at the end of 120 hours.

With the lytic filtrate III slight turbidity was also seen in the tubes at dilutions of 10^{-22} to 10^{-26} after 24 hours. The cultures cleared after 48 hours and remained unchanged 72 hours after inoculation. At the end of 120 hours there was also slight turbidity of the culture with a few clumps of agglutinated bacteria.

In both series of cultures at dilutions of 10^{-22} to 10^{-26} turbidity increased after 144 hours until at the termination of the experiment there was practically the same amount of growth as in the check tubes. At dilutions of 10^{-27} to 10^{-50} no lytic action was shown by either filtrate, the growth of the cultures at each reading being identical with that of the untreated check tubes.

At the higher dilutions 10^{-21} to 10^{-25}, inclusive, it seems possible that, in view of the corpuscular nature of the bacteriophage, the preponderance of *Ps. tumefaciens* over the quantity of the lytic agent might account for the continued growth of some of the bacteria. After standing 144 hours poured plates were made of the cultures in lytic filtrate V at a dilution of 10^{-22} by adding two cc. of the filtrate to 30 cc. of crystal violet bile agar. After six days the three plates showed only 47 colonies of the crown-gall organism. In the plates from the untreated check tubes, using two three-millimeter loops of the inoculum to the same amount of agar, the colonies of *Ps. tumefaciens* were too numerous to count.

INACTIVATION OF THE LYTIC AGENT IN BILE AGAR CULTURES

In attempts to demonstrate the formation of plaques in cultures of *Ps. tumefaciens* by lytic principles III and V, the organisms was streaked upon agar poured plates and a drop of the lytic principle placed in the center of the streak. As bile crystal violet agar has been found to be an excellent medium for the growth of the crown-gall organism, this medium was employed in the above tests. However, it was found that the lytic filtrate when applied to the organism on this medium failed to form plaques, that is, failed to lyse *Ps. tumefaciens*.

Having previously demonstrated that the lytic filtrate of this passage prevented growth of the pathogene in broth cultures, further trials were made with streaks of the organism on plates of neutral potato-dextrose and neutral bile crystal violet agar. The plates were examined two days after streaking and the application of the lytic principle. As checks the streaks

of the organism on agar plates were not treated with the lytic principle. In every case the application of a drop of the lytic principle to the streak culture of the organism on neutral potato-dextrose agar caused lysis, while in the bile agar plates there was no lytic action. These results are in accord with those of d'Hérelle (9) who showed that bile exerts an inhibitory action upon the Shiga bacillus bacteriophage but does not cause its destruction.

VARIATION IN SUSCEPTIBILITY OF PSEUDOMONAS TUMEFACIENS TO THE ACTION OF THE BACTERIOPHAGE

The plate cultures made from lytic filtrate V at a high dilution, to which *Ps. tumefaciens* had been added, indicated that certain individual bacteria within the culture might be more resistant than others to the action of the lytic agent. The phenomenon of resistance and susceptibility to bacteriophage has been demonstrated by d'Hérelle (9), Gerretsen, Gryn, Sack, and Söhngen (5), Coons and Kotila (3), and others. Attempts, therefore, were made to determine whether there was any morphological difference between the colonies of *Ps. tumefaciens* which were resistant and those susceptible to the lytic agent. Using bile agar, dilution plates were made from certain lytic filtrate cultures which showed no turbidity at dilutions of 10^{-3} to 10^{-5} after having been exposed to the bacteriophage for seven days.

Only a few colonies came up on the plates and these were of the rough type, i.e., showing striations of the surface growth and with serrate margins. Plate cultures were also made from the lytic filtrate at dilutions of 10^{-17}, 10^{-15}, and 10^{-30}, which showed moderate turbidity after seven days. There was an abundant colony development on all these plates. The colonies in these cases were typical of the smooth type with serrate margins. Subcultures were made from the smooth and rough type colonies and these, after 24 hours growth in broth, were used to inoculate young tomato plants and also lytic filtrates III and V at dilutions of 10^{-1} to 10^{-20}, inclusive. There was no inhibitive action shown by the filtrates and galls developed on the inoculated plants. These results suggest that certain of the bacteria may be resistant to the action of the bacteriophage as shown by d'Hérelle (9) and, in such cases, growth is inhibited but complete lysis does not take place.

TEMPERATURE RELATIONS

Coons and Kotila (3), working with a polyvalent bacteriophage from decaying carrot, showed that incubation at 36.1° C. for six days greatly decreased the lytic action against *B. carotovorus*. Mallman and Hemstreet (13) showed that their inhibitory substance, isolated from a decaying cabbage, was killed by exposure of 30 minutes to a temperature of 63° C.

Tests were made to determine the effect of high temperature upon bacteriophage V. In these tests, three tubes containing 2 cc. of lytic principle diluted one to one with alkaline broth were suspended in a water bath with a fourth broth tube in which was placed a thermometer. By constant stirring, the temperature was held at a maximum variation of one degree. After the liquid in the thermometer tube had reached the required temperature, it was held for ten minutes. Usually only two minutes were necessary to raise the temperature in the tubes from 25° C. to that required. After heating the tubes containing the lytic agent they were cooled at 25° C. and 0.1 cc. of a 24-hour broth culture of *Ps. tumefaciens* added. Preliminary tests were made at temperatures of 45, 50, 55, 60, 65, 70, and 75° C. After cooling, the lytic principle was diluted to 10^{-2} in sterile broth and one drop of a 24-hour broth culture of *Ps. tumefaciens* added. Readings were made each 24 hours until 96 hours had elapsed. The diluted, heated filtrates at all temperatures showed very slight clouding of the liquid after 48 hours. At this time a few clumps of the agglutinated bacteria were also present as a precipitate in the tubes. However, after 72 hours the turbidity disappeared in every case with an increase in the masses of agglutinated organ-

TABLE 5.—*Effect upon lytic filtrate V of heating for ten minutes at different temperatures*[a]

Culture method	Temperature; degrees C.								Check not heated
	60	70	75	80	85	90	95	98	
A	0	0	0	1[b]	4	4	4	4	4
B	0	0	0	1[c]	4	4	4	4	4
C	0	0	0	1[d]	4	4	4	4	4

[a] Culture methods A, B, and C refer to the methods 1, 2, and 3, as given in the text immediately preceding the table. Growth is indicated as follows:

[b] One tube showed no growth; one tube showed slight turbidity with masses of agglutinated bacteria and one tube with abundant growth.

[c] Two tubes showed no growth; one tube showed slight turbidity with a few precipitated clumps of bacteria.

[d] Two tubes showed no growth; one tube showed slight turbidity with clumping of the bacteria.

0 = No growth.
1 = Very slight growth.
2 = Slight growth.
3 = Moderate growth.
4 = Abundant growth with pellicle formation.

isms. The results of this test showed that a temperature of 75° C. for 10 minutes did not inactivate the lytic principle; therefore, further trials at higher temperatures were made. The following trials at temperatures of 60, 70, 75, 80, 85, 90, 95, and 98° C. were made in triplicate, employing the same method as given previously.

The effect of heating upon the lytic principle was tested in three sets of cultures by the following methods: (1) One drop of the heated lytic principle was added to a 10 cc. 24-hour-old broth culture of *Ps. tumefaciens*; (2) one drop of the lytic principle and one drop of inoculum were added to 9 cc. of sterile broth; (3) 2 cc. of the lytic principle were added to an equal volume of a 24-hour-broth culture of *Ps. tumefaciens*. Final readings of the cultures were made after 168 hours.

The summarized results of the three trials are presented in table 5.

From the data presented in table 5 it is evident that the lytic principle was completely inactivated by heating for 10 minutes at 85° C. At a temperature of 80° C. for 10 minutes, although the lytic principle was not able to prevent growth of the crown-gall organism in every tube (see footnote of table), there was still evidence of inhibition to bacterial growth. The results of these tests show that the temperature causing inactivation of the lytic principle V lies between 80° and 85° C.

Previous experiments having shown that lytic filtrate V in high dilutions would inhibit the growth of *Ps. tumefaciens*, the question arose as to whether or not the filtrates would be effective against phytopathogenes other than the crown-gall organism. It was also of interest to know whether this filtrate would likewise inhibit the growth of *Ps. tumefaciens* isolated from other hosts or from the same host at various times. Although the valency of lytic principles had been determined by other investigators, no tests employing the same pathogene from numerous isolations had been made.

SPECIFIC ACTION OF THE LYTIC PRINCIPLE

Coons and Kotila (3) and Mallman and Hemstreet (13) showed that the bacteriophages which they isolated from decaying carrot and cabbage, respectively, were polyvalent. Gerretsen, Gryn, Sack, and Söhngen (5) found the bacteriophage from legume nodules monovalent and specific for certain strains of *B. radicicola*. Tests, therefore, were made to determine the valency of lytic principle V and the specificity of its action upon *Ps. tumefaciens* isolated at different times from various hosts.

After fourteen successive passages through a Berkefeld W filter, the lytic filtrate V in dilutions of 10^{-1} to 10^{-9}, inclusive, was tested against the isolation of *Ps. tumefaciens* from which the bacteriophage was isolated, *Ps. pruni, Ps. beticola* (Smith, Brown, Town.) Potebnia, *Ps. citri* Hasse,

Ps. vesicatoria Doidge, *Ps. phaseoli* E. F. S., *B. carotovorus,* and *B. atrosepticus.* Cultures of these pathogenes had been inoculated previously into their respective hosts and their pathogenicity proved.

After making the required dilution of the lytic principle in 10 cc. of neutral broth, two drops of a 24-hour-broth culture of the organism to be tested were added to each tube. The results of this trial after four days growth are given in table 6.

TABLE 6.—*Effect of lytic principle V upon Pseudomonas tumefaciens and seven other plant pathogenes*[a]

Organism	Growth response at lytic filtrate dilutions								Check
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	
Ps. tumefaciens	0	0	0	0	0	1–	1–	1–	4
Ps. pruni	2	2	2	2	2	2	2	2	2
Ps. beticola	3	3	3	3	3	4	4	4	4
Ps. citri	2	2	2	2	2	2	2	2	2
Ps. vesicatoria	2	2	2	2	2	2	2	3	3
Ps. phaseoli	2	2	2	2	2	2	2	2	2
B. atrosepticus	3	3	3	3	3	3	3	3	3
B. carotovorus	4	4	4	4	4	4	4	4	4

[a] Growth is indicated as follows:
0 = No growth of the organism, broth clear.
1– = Very faint haze but less growth than 1.
1 = Very slight growth, very faint turbidity of broth.
2 = Slight growth, faint turbidity of broth.
3 = Moderate growth, slight turbidity of broth.
4 = Abundant growth with pellicle formation.

From table 6 it is seen that lytic principle V affected only the culture of *Ps. tumefaciens,* the other organisms growing as abundantly in the presence of the bacteriophage as in the untreated check cultures. At dilutions of 10^{-7} to 10^{-9} there was only a perceptible clouding of the cultures of *Ps. tumefaciens* with a few clumps of agglutinated bacteria, showing that the lytic principle exerted an inhibitory effect upon the organism although lysis was not complete.

Similar tests were also made with the same organisms, using lytic filtrate V after 16 passages through the Berkefeld W filter. In these trials complete lysis of *Ps. tumefaciens* took place at all dilutions 10^{-1} to 10^{-9}, inclusive, while the other plant pathogenes were not affected.

TABLE 7.—*Specific action of lytic principle V in different dilutions against Psudomonas tumefaciens isolated from different hosts*[a]

Number of culture	Source	Growth response at lytic filtrate dilutions						Check
		1–20	1–200	1–2,000	1–20,000	1–200,000	1–2,000,000	
1110	Apple	4	4	4	4	4	4	4
1162	Rose	4	4	4	4	4	4	4
R	Raspberry	0	0	0	0	0	1–	4
1212	Geranium	2	2	3	3	3	3	4
1215	Apple	4	4	2	3	4	4	3
1216	Apple	2	3	2	3	3	3	3
1217	Peach	3	3	3	4	4	4	4
1218	Apple	3	3	3	4	4	4	3
1219	Peach	4	4	4	4	4	4	4
1220	Walnut	3	3	3	2	2	2	3
1221	Apple	4	4	4	4	4	4	4
1223	Rose	2	2	2	2	2	2	2
1224	Weeping willow	4	4	4	4	4	4	4
1225	Western Sand cherry	4	4	4	4	4	4	4
1226	Raspberry	4	4	4	4	4	4	4
1227	Apple	4	4	4	4	4	4	4
1228	Incense cedar	4	4	4	4	4	4	4

[a] Growth is indicated as follows:

0 = No growth of the organism, broth clear.
1– = Doubtful growth.
1 = Very slight growth, very faint turbidity of broth.
2 = Slight growth, faint turbidity of broth.
3 = Moderate growth, slight turbidity of broth.
4 = Abundant growth, with pellicle formation.

Having shown that lytic principle V was univalent in its action when tested against seven other bacterial plant pathogenes, further trials were made using 15 isolations of the crown-gall organism from various hosts or from the same host at different times.

In this experiment lytic principle V was used after seven, eight, and nine successive passages through a Berkefeld W filter. A summarized record of the experiment at the end of four days is given in table 7.

The only culture which showed complete inhibition of growth was that of strain R (raspberry) from which lytic principle V was isolated. Culture 1226, a strain of the pathogene also isolated from a crown gall on a different raspberry plant, was not affected by the lytic principle. This failure of the lytic principle to affect culture 1226 indicates the specificity of the principle for the strain of the pathogene from which the bacteriophage was isolated. Further, the lability of the crown-gall organism is shown by the failure of the lytic principle to cause lysis of culture 1224. This latter culture was isolated from a six-months-old gall on weeping willow induced by inoculation with culture R, the strain from which lytic principle V was isolated. Such reaction indicates that the host may exert some influence upon the strain of the organism to change its reaction to the lytic principle.

LONGEVITY OF THE LYTIC AGENT IN CULTURE

Having shown that lytic filtrates III and V at dilutions of 10^{-21} prevented growth of *Ps. tumefaciens,* the question arose as to how long the filtrates would remain active without further addition of young cultures of the crown-gall bacteria and subsequent filtrations. Portions of filtrates III and V after the nineteenth passage through the Berkefeld filters were set aside on November 1. On January 20, dilutions of the filtrates in a series of 10^{-1} to 10^{-10} were made and one drop of a 24-hour broth culture of *Ps. tumefaciens* added to each tube. There was abundant growth in all of the tubes containing filtrate III, but no evident growth in filtrate V at the dilutions 10^{-3} and below. After two additions of young cultures of the crown-gall organism and subsequent filtrations, the potency of lytic principles III and V was again tested in a similar manner. At the dilution 10^{-5} no growth was evident four days after the addition of one drop of a 24-hour broth culture of *Ps. tumefaciens* to 5 cc. of the lytic filtrate culture. In subsequent trials made February 16 and March 3, there was only a very slight inhibition of growth of *Ps. tumefaciens* in both the stock filtrates. In these trials, the clouding of the culture was less than in the check broth tubes, and, in addition, there was a noticeable clumping of the bacteria 48 hours after adding one drop of the bacterial suspension to the filtrate. There was also a slight agglutination of the crown-gall bacteria at this time

in 1–10 dilutions of the filtrates. Growth at this dilution was almost as abundant as in the check tubes with the exception that no pellicle was formed. From these trials it appears that the bacteriophage does lose its potency upon prolonged standing in culture (about two and one-half months).

SUMMARY

A bacteriophage has been isolated from nonviable cultures of *Pseudomonas tumefaciens* (raspberry isolation) in sterilized and nonsterilized soil, from a virulent culture of the same isolation of the organism in broth, and from a crown gall on sugar beet. In high concentrations the lytic principle from all of these sources has caused complete lysis of *Ps. tumefaciens* in broth cultures.

Cultures were made by adding 1 cc. of a 24-hour broth culture of *Ps. tumefaciens* to 1, 2, 3, 4, and 5 cc., respectively, of lytic filtrate III after 14 filtrations. No infection resulted on young tomato plants from inoculations of the five cultures after being subjected to the action of the lytic agent nine hours or longer.

Injections of the lytic filtrate of known potency into young tomato plants failed to prevent infection when the pathogene was inoculated into the plant by means of the puncture originally made by the hypodermic needle.

The potency of the lytic agent was enhanced by additions of young broth cultures of *Ps. tumefaciens* to the stock filtrate along with successive filtrations through Berkefeld W filters. After 19 passages there was no evidence of growth of *Ps. tumefaciens* when added to dilutions 10^{-20} and 10^{-21}, respectively, of filtrates III and V. At higher dilutions growth was inhibited for 72 to 120 hours after inoculation but later became abundant.

The rough type of *Ps. tumefaciens* was recovered in poured plates from broth cultures of the lytic filtrate at dilutions of 10^{-3} to 10^{-5}, which showed no evidence of growth seven days after inoculation. Subcultures of the rough-type colonies were inoculated into other broth dilutions of the filtrate. From these cultures, showing moderate turbidity after seven days, *Ps. tumefaciens*, having the smooth-type colony, was plated out.

The potency of the lytic filtrate is greatly reduced by heating for ten minutes at 80° C. and completely inactivated at 85° C. for the same length of time. The lytic action of the bacteriophage is inhibited by streaking on bile agar.

The lytic agent when tried against *Ps. pruni, Ps. beticola, Ps. citri, Ps. vesicatoria, Ps. phaseoli, B. atrosepticus, B. carotovorus,* and *Ps. tumefaciens* inhibited growth of the crown-gall organism alone.

In trials using 17 strains of *Ps. tumefaciens,* the lytic agent affected only the culture of the organism from which it was isolated.

The lytic agent V tried against cultures of *Ps. tumefaciens,* R and 1226, isolated at different times from separate raspberry galls, affected only the former culture from which the bacteriophage was isolated. Culture 1224, a reisolation of culture R after passage through weeping willow, was not affected by lytic filtrate V.

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THE EFFECTS OF POTASH AND PHOSPHORUS ON TIP BURN AND MILDEW OF CABBAGE

CHARLES CHUPP

Fully ten years ago the writer was called into Monroe County, New York, to diagnose a certain trouble on cabbage. The plants in a third of a field had nearly every leaf showing dead, brown or black margins, with the lower leaves fairly well spotted by Alternaria. In the most pronounced cases whole plants were somewhat dwarfed and loose of head. The only difference in treatment to which the affected cabbage was subjected was a rather heavy side dressing with nitrate of soda. At that time the question arose as to whether the nitrate caused the plants to be more susceptible to Alternaria, which in turn might indirectly produce the tip burn effect.

Since then a similar injury was observed in many Danish Ballhead cabbage fields, but only occasionally on the early and kraut varieties. It soon was determined to be worse some years than others, and on some farms than others. It seemed more prevalent when fall-plowed soil became too hard in the spring to be made friable by cultivating tools or when the ground was puddled in the spring when it was plowed. There seemed to be no correlation between the amounts of the trouble on high ground and low ground, although at times it was noticeable that the sloping land gave rise to more tip burn than did the level stretches in the same field. Neither did acidity nor alkalinity seem to play an important part in the production of the malady.

All of these observations, together with the fact that some of the best growers maintained that they could not grow Danish cabbage after turning under a heavy alfalfa or clover crop, led to the conclusian that the tip burn was the direct result of too large an application of nitrogen and that possibly the addition of a certain amount of superphosphate (acid phosphate) would counteract the supposed injurious effects of the nitrogen. It was some time before this supposition could be tested under field conditions, but, as the tip burn became more of a problem, and when the growers insisted that help was necessary if successful growing of the late crop was to be continued, experiments were undertaken in 1927. These dealt only with superphosphate and nitrate of soda. Not even a check without fertilizer could be included, as Mr. E. N. Reed of Cortland County, the grower who kindly had consented to cooperate, had treated all of his field with the superphosphate. Therefore, the only thing undertaken was to apply to part of the field a double amount of the superphosphate, then divide the area into plots on some of which no nitrate was added, on others the same

amount that Mr. Reed usually applied for cabbage, and on still others twice the amount of nitrate. One plot had three times as much nitrate of soda as was the usual application on this farm. All the other plots were replicated seven times. Each plot consisted of four rows of 20 plants each.

The arrangement of the plots was faulty, since all the double amounts of superphosphate were on one half of the experimental field and those with single applications on the other half. Fortunately, however, the regular part of the field was quite uniform in its amount of tip burn. The results, therefore, have some value in determining the effects of sodium nitrate and superphosphate on the prevalence of the disease.

TABLE 1.—*Effects of phosphorus and nitrogen on tip burn in 1927. Reed farm*

Pounds of fertilizer applied per acre		Percentage of tip burn
16 per cent superphosphate	Nitrate of soda	
1250	166	27.78 ± 1.104
1250	332	17.22 ± 4.924
1250	None	27.22 ± 6.389
625	166	12.78 ± 3.408
625	332	12.44 ± 4.285
625 (One plot)	498	17.00 ——
625	None	9.44 ± 0.613

The results were unexpected, for the percentage of tip burn was increased rather than lessened where the amount of superphosphate was doubled. It also is true that where the amount of sodium nitrate was tripled the increased tip burn was very noticeable, but, where it was merely doubled, the amount of tip burn was not nearly so great as it was in the doubled superphosphate plots.

The question then arose in regard to the importance of each of these two fertilizer ingredients in the production of tip burn and just what effect potash would have alone or in combination with either one of them. Previous work by Newhall[1] suggested that potash or nitrogen may increase the severity of tip burn in lettuce. Would it do the same in the case of cabbage? It was remembered, however, that the tip burn in lettuce begins on the younger, more tender leaves, and spreads outward to the older ones, while, in cabbage, the opposite is true, and that only when the trouble is severe are the inner leaves injured enough to reduce the size of the plant and resulting head. Might not, therefore, the reaction of cabbage to potash differ from that of lettuce?

[1] Newhall, A. G. Studies on the tip burn disease of lettuce. Phytopath. 15: 58. 1925.

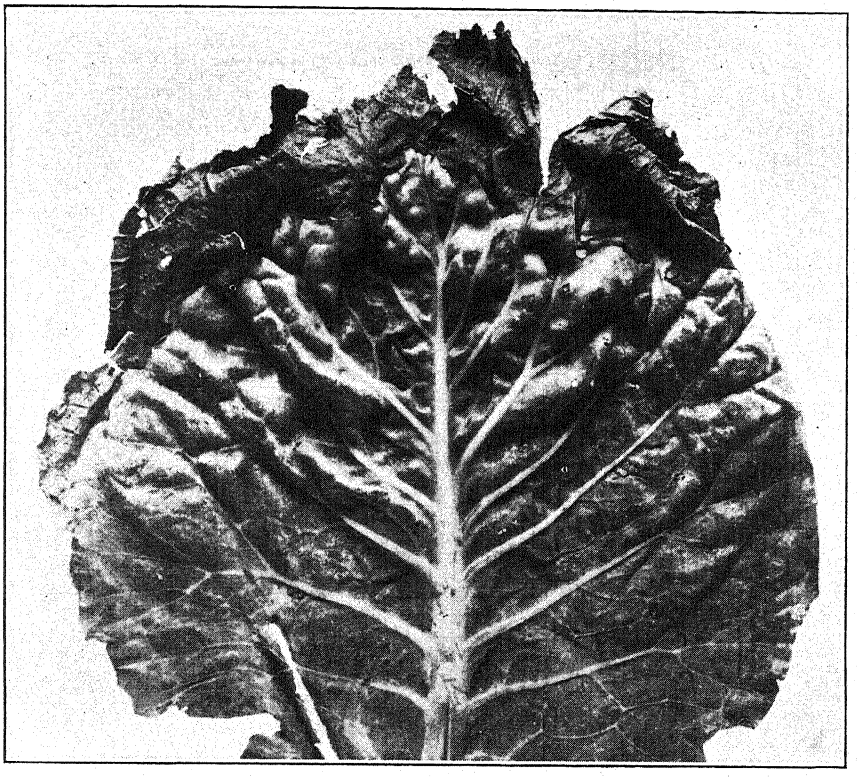


FIG. 1. Tip burn on a cabbage leaf.

In 1928 rather detailed experiments were made on the Reed farm, Cortland County, and on the George Maier farm, Monroe County, to test more fully the effects of the various fertilizer elements on the disease. Incidently, the Maier farm was one where tip burn had become so troublesome that it was being considered unprofitable to grow cabbage for storage. The yield was reduced and the harvested cabbage had to be either trimmed too closely for good keeping in storage or left with tip burned leaves from which secondary rots spread to various depths in the head.

TABLE 2.—*Arrangement of the plots on the Maier farm, 1928*

N–PP–K	P–N	P–K	P–P	P	N–PP–K	P–N	P–K	P–P	P
N–P–KK	K–N	K–K	P–K	K	N–P–KK	K–N	K–K	P–K	K
NN–P–K	N–N	K–N	P–N	N	NN–P–K	N–N	K–N	P–N	N
N–P–K	N	K	P	O	N–P–K	N	K	P	O

P = Acid phosphate, N = Nitrate of soda, K = Potash, O = Check.

The arrangement on the Reed farm was similar, but had no complete fertilizer included. The plots in each of the experiments consisted of four rows and twenty plants in each row. There were not so many checks nor so large a number of replications as was desired because of limited space.

The soil on the Reed farm was a Lordstown slit loam and on the Maier farm, Ontario fine sandy loam. On both places the soil was in excellent condition, having in the past years received heavy legume cover crops, some barnyard manure, and a definite amount of commercial fertilizer. The cabbage crop in both cases was very good, except for some tip burn, especially on the Maier farm.

TABLE 3.—*Effects of fertilizer on tip burn in 1928*

Treatment	Percentage of tip burn	
	Reed farm	Maier farm
N–PP–K	———	34.04 ± 11.357
PP	46.83 ± 8.792	13.63 ± 1.810
P	38.32 ± 9.621	26.62 ± 9.172
PN	16.78 ± 2.876	26.88 ± 10.269
NN	1.05 ± 0.355	18.42 ± 2.456
N	2.44 ± 0.644	17.54 ± 2.507
PK	7.79 ± 1.922	7.73 ± 2.798
NN–P–K	———	7.16 ± 0.161
N–P–K	———	5.87 ± 1.604
KN	1.25 ± 0.326	6.96 ± 1.189
K	4.83 ± 1.805	9.44 ± 2.04
KK	1.56 ± 0.913	9.34 ± 3.241
N–P–KK	———	3.70 ± 0.608
0 (Check)	9.98 ± 3.545	15.72 ± 6.178

PP, NN, KK = Double amounts of these materials.

The applications in all cases, throughout the three years, were made after the plants were set into the field, excepting the first year, when the superphosphate was applied before the planting. The material for each individual plot was weighed separately and strewn over the soil by hand as uniformly as possible. A cultivator was then used to cover the fertilizer. On the Reed farm 333 pounds of nitrate of soda, 625 pounds 16 per cent superphosphate, and 200 pounds of muriate of potash (approximately 5–8–8 formula) were applied on the acre basis. In the case of the Maier farm the number of pounds was 80, 220, and 40, respectively (approximately 3–10–6 formula).

Counts of tip burn were made late in September. It was difficult to obtain accurately the amount of the disease present. At first an attempt

was made to count each tip burned leaf. This was very laborious and, besides, did not give a true representation, for some of the most seriously affected plants had fewer leaves because of the disease. Therefore, it was finally decided to divide the degree of infection into three classes: Mild, medium, and severe. Then, in getting the percentage, the number under severe was multiplied arbitrarily by three and that under medium by two. The sum of these was added to the figure under mild. Thus the figures in the tables would represent or be equivalent to the given percentage of mild tip burn. At least, the method gave uniformity in measuring the amount of injury and seemed better than merely counting the affected plants. The data were collected without consulting the copied plan of the experiment, so that the judgment would not be biased in making the counts. In addition, other workers were asked to aid in the checking, to insure greater accuracy in the results.

As might be supposed by working with anything as variable in its composition as the soil, there was much variation in the percentages obtained. This was true particularly because, on the Reed farm, there was only a medium amount of rainfall, while there was more of it on the Maier farm. In consequence, the nitrogen seemed to reduce injury in the drier field and had little or no effect in the wetter one. These results with nitrogen were all the more striking, since, the following year, the condition in the two counties was just reversed. Still, nitrogen apparently reduced the amount of tip burn in the dry soil and had little effect where there was more rainfall. It is for this reason that the combined figures for the three seasons are too variable in the case of nitrogen to say definitely what the results will be for any given year.

Notwithstanding the variability in results from nitrate of soda, the effects from superphosphate and muriate of potash were surprisingly uniform whether the soil was relatively wet or dry. Superphosphate, when used without potash, always increased the amount of tip burn. Potash always decreased it. For instance, single applications of superphosphate in 1928 gave 38 and 27 per cent of tip burn, while the plots without fertilizer gave 10 and 16 per cent and the single potash plots gave 5 and 9 per cent. The double superphosphate plots on the Maier farm gave a much smaller percentage increase than was expected, but PP plots gave 47 and 14 per cent, respectively, on the two farms, in contrast to the 2 and 9 per cent of the KK plots.

In 1929 the experimental plots were discontinued on the Reed farm, because the method of fertilizing seemingly furnished so nearly a properly balanced ration for the soil that tip burn was not serious enough to warrant the drawing of conclusions. Mr. Claude Rorapaugh of Cortland, who rented a farm quite depleted in plant food, kindly consented to act as a

cooperator. He permitted the use of an area large enough to supply sixty-four plots, of four rows each and thirty-seven plants in the row. The remainder of his field had an application of 600 pounds an acre of 16 per cent superphosphate and 300 pounds of a 10–10–10 mixture. The soil was a Chenango stony silt-loam type, which grew a large yield of cabbage. The experiment was continued on the Maier farm in Monroe County, although in another field. There also were sixty-four plots of four rows each with twelve plants to the row. A portion of the remainder of the field had 450 pounds of 5–10–5 fertilizer and another portion 200 pounds of 0–12–18. The Rorapaugh farm had plenty of rain, but on the Maier farm there was almost none after the cabbage was set out.

TABLE 4.—*Arrangement of plots on Maier and Rorapaugh farms, 1929*

P	K	N	O	KN	N	PN	NN	KN	PK	K	KK	P	PN	PK	PP
PN	KN	NN	N	PK	P	PP	PN	N	P	O	K	K	KN	KK	PK
PK	KK	KN	K	K	O	P	N	PN	PP	P	PK	N	NN	PK	PN
PP	PK	PN	P	KK	K	PK	KN	NN	PN	NO	KN	O	N	K	P

On both farms the single applications of the fertilizing elements were the same as represented in a thousand pounds per acre of 5–10–5. Where the

TABLE 5.—*Effects of fertilizer on the amount of tip burn on the Maier and Rorapaugh farms in 1929*

Treatment	Percentage of tip burn	
	Maier farm	Rorapaugh farm
PP	37.95 ± 4.263	5.70 ± 0.826
P	30.82 ± 5.601	7.21 ± 1.451
(5–10–5) (450 lbs.)	26.00 ± 3.028	———
PN	15.74 ± 2.305	8.86 ± 2.610
O (Check)	11.17 ± 3.032	1.04 ± 0.375
PK	10.92 ± 2.350	1.36 ± 0.477
K	6.29 ± 1.702	0.17 ± 0.108
N	4.16 ± 0.952	1.65 ± 0.604
KK	5.15 ± 1.504	0.00
KN	2.54 ± 0.556	0.21 ± 0.132
NN	1.62 ± 0.287	0.37 ± 0.215
(10–10–10) (+ 600 lbs. P)	———	2.50 ± 0.859
(0–12–18) (200 lbs.)	0.20 ± 0.224	———

TABLE 6.—*Summary of experiments on the effects of potash and phosphorus on the tip burn of cabbage*

Treatment of plots	Order of tip-burn severity in the different experiments													
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th
PP	2	1	1				1							
N–PP–K	1													
PPN	1													
PN	1	1	1	1										
P		3	1			1								
PPNN			1											
O (Check)			1	2		1	1							
NN				1				1			2			
10–10–10 + 600 lbs. P.				1										
N					2			2						
PNN					1									
PK					2					1				
5–10–5						2								
K							2	1		1				
KK									3		1			
KN									1	2		1		
NN–P–K											1			
0–12–18												1		
N–P–K													1	
N–P–KK														1

letters are doubled a double portion of this element was applied. The material was strewn as uniformly as possible by hand after the plants were set into the field and covered with the cultivator. The results in 1929 were substantially the same as those obtained in 1928. The effects of the nitrogen again were variable, but, in general, seemed to reduce the amount of tip burn. The superphosphate increased the amount of tip burn, and potash decreased it, as is shown by table 5.

In none of the three years while the experiments were being conducted did the chosen fields show much tip burn. For this reason some of the probable errors are too large to make the figures significant. But, wherever tip burn was at all abundant it was easy to recognize a superphosphate plot from that which received potash. This is brought out fairly clearly when all the data are summarized in one table.

The difference may be represented also by averaging the percentages of the five experiments.

TABLE 7.—*Tabulated summary of the results of three years' experiments on the effects of potash and phosphorus on tip burn of cabbage*

Treatment of plots	No. of experiments	Percentage of tip burn
PP	5	26.266 ± 4.525
P	5	22.481 ± 3.039
PN	5	16.210 ± 1.810
NN	4	5.365 ± 1.774
KK	4	4.01 ± 1.214
K	4	5.18 ± 1.126
KN	4	2.74 ± 0.867
0 (Check)	4	9.48 ± 1.794

In 1928 Quanjer[2] mentions a case where cabbage grown in experimental plots deficient in potash had much tip burn. Cauliflower, also, showed harmful effects under the same conditions, but the symptoms were of a different nature. In the plots where the potash was supplied both crops were healthy, thereby showing that the lack of potash was the cause of the trouble. In a still more recent publication Schoevers[3] illustrated currant leaves that were dwarfed and tip-burned where potash was lacking and showed the healthy branches from plots where this mineral was supplied. It seemed that the form potassium sulphate was more desirable than potassium

[2] Quanjer, H. M. De invloed van kaligebrek op de vat baarheid van bloemkool voor *Peronospora parasitica*. Tijdschr. Plantenziekten 34: 254–256. 1928.

[3] Schoevers, T. A. C. Een proef met zwavelzure kali tegen "Randjesziekte" bij roode bessen. Tijdschr. Plantenziekten 35: 231–233. 1929.

chloride. Many other cases in literature could be cited where some form or ingredient of fertilizer was capable of changing the immunity or the susceptibility of host plants to various types of diseases. Some of these cases have been proved beyond doubt, while many others are little more than conjectures based on very meager experimentation.

The data given in this discussion may not seem complete enough to justify much generalizing, but the results of the experiments, together with many observations, have persuaded the writer that the farmers have at hand a means of controlling their cabbage tip burn. On a number of farms a fertilizer high in potash, as 5–8–7, was used successfully in the control of the trouble and also induced excellent yields. The formula 4–12–4, used on some farms, increased tip burn wherever the disease was present. In one community, where the growers still clung to the old-time formula of 2–8–10, it was very noticeable that the late cabbage was not affected by tip burn. This statement is not meant as a recommendation of such a formula but is given merely to show that fertilizer with a high potash analysis sometimes is desirable.

No attempt was made to determine the reason why lack of potash or an increase of phosphorus causes tip burn; but in a talk with a plant physiologist it was suggested that potassium is in some way connected with the respiration processes and is found most abundantly in the meristematic tissue. In a plant depleted of this mineral, the potassium may be withdrawn from the older tissues and localized in the meristematic areas. On the other hand, an increase in phosphorus, an element necessary to cell division, might tend to increase the growing area and thus hasten the potassium withdrawal from the leaves. Because of the consequent incomplete respiration, this would be followed by the presence of toxic substances that might cause the dying of the leaf tissue.

No plot yields are given because, where single elements were used, the yield necessarily was reduced. Furthermore, no attempt was made to get the combination of fertilizer that would produce the highest yield. That is a subject for the agronomist and not for the pathologist.

Quanjer,[4] in the article previously quoted, discussed the effect of potash on the presence of downy mildew of cabbage and cauliflower (*Peronospora parasitica* (Pers.) de Bary). He came to the conclusion from his observations that downy mildew was much more severe in the plots lacking potash. He mentioned also work that had been done on *Plasmopara viticola* and *Bremia lactucae* in which quite similar results were obtained. He is inclined to believe that all downy mildews might react in the same manner.

[4] *Loc. cit.*

The fertilized plots for tip burn in 1929 had a fairly large amount of downy mildew on the leaves and, therefore, afforded an opportunity for obtaining information regarding the influence on this disease. The data obtained in the two counties are given below.

TABLE 8.—*Effects of fertilizer on the downy mildew of cabbage*

Treatment	Average number of mildewed leaves in the plots	
	Rorapaugh farm 74 plants counted per plot	Maier farm 48 plants per plot
KK	72.345 ± 2.704	14.325 ± 2.533
K	71.446 ± 5.389	12.525 ± 1.449
PK	56.031 ± 7.150	11.700 ± 3.260
KN	53.449 ± 5.527	16.037 ± 2.314
NN	43.360 ± 6.961	11.725 ± 1.487
N	40.714 ± 2.989	19.950 ± 2.717
PN	31.939 ± 4.580	19.875 ± 4.333
O	28.347 ± 1.910	11.175 ± 3.032
P	25.647 ± 6.113	5.237 ± 0.722
PP	19.910 ± 2.561	1.825 ± 0.580
0–12–18	.....	22.600 ± 2.239
5–10–5	.....	22.000 ± 1.780
(10–10–10) (+ 600 P)	14.292 ± 1.275	.....

It was less difficult to record the amount of mildew than of the tip burn. The number of spots on a leaf was correlated very closely with the number of affected leaves per plant. Therefore the record was taken by counting the number of affected leaves in each plot.

The information at hand is insufficient to permit drawing conclusions, especially since the figures for the Maier farm are so variable. But the results of the one year seem to be diametrically opposed to the observations of Quanjer. It would be interesting to follow this with at least two more years of experimentation to prove more fully the effect of the different fertilizer ingredients on the downy mildew. Since this is not possible at the present time, these observations and figures are given with the hope that some one else will study the subject more fully.

When recording the data the potash plots were evident because there was little tip burn and much downy mildew. The superphosphate plots could be recognized because of just the opposite results. The nitrogen also seemed to increase the mildew. This was true both on the Maier farm where rainfall was almost entirely lacking and on the Rorapaugh farm where the rainfall was abundant. On the latter farm potash very evidently increased mildew, while on both farms phosphorus decreased it. A table

giving the odds in comparing some of the plots having potash with those having superphosphate will give something regarding their relative importance.

TABLE 9.—*Odds as obtained by the "Student's" Table in comparing some of the mildew plots*

Treatment of plots	Plots with which compared	Odds showing relative significance of difference	
		Rorapaugh farm	Maier farm
KK	PP	1249 to 1	16 to 1
K	P	2499 to 1	293 to 1
PK	PN	28 to 1	1 to 4
KN	PN	97 to 1	1 to 2
NN	O	3 to 1	1 to 2
K	N	2499 to 1	1 to 3
O	PP	4 to 1	3 to 1
KK	O	4999 to 1	1 to 1

There was almost three times as much mildew on the Rorapaugh farm as on the Maier farm which lacked the summer rains. This aids in making the odds insignificant on the latter place. But on both farms there is evidence to show a significant difference between the K and P plots and even the KK and PP ones, although the results from the double applications were not so consistent as were those from the single amounts of potash and phosphorus.

As was stated above, no conclusions may be drawn from the two experiments. But, even if potash were found to increase downy mildew, the fact would be of little economic importance; for, under New York State conditions, mildew never is serious enough in the field to cause any reduction in yield. Therefore, the recommendation that a fertilizer formula with a larger percentage of potash be employed in combating tip burn can still be followed with no fear of increasing another trouble. The only place where downy mildew might ever be of consequence is in the seed bed, so that if the data on the Rorapaugh farm later prove correct the seed bed could be protected from mildew by a heavy application of superphosphate and a correspondingly light one of potash.

SUMMARY

Tip burn of cabbage is fairly general and sometimes severe on Danish Ballhead cabbage grown in New York State. The trouble rarely is found on kraut and early varieties. Heavy applications of superphosphate (acid

phosphate) increased the amount of tip burn, especially where nitrogen and potash were lacking or were present in small amounts.

Nitrate of soda had little effect for or against the production of tip burn. It seemed to increase the disease when the applications were heavy.

Muriate of potash reduced the amount of tip burn enough to recommend it as a practical control measure. The fertilizer ratio 1–2–2, as represented by the formula 5–8–7 or similar ones, is approximately the correct one on the farms where the experiments were conducted.

Downy mildew of cabbage was present very prominently on one farm where the tip-burn experiments were conducted, and a small amount on the second farm.

Preliminary experiments indicate that potash and nitrogen may increase the amount of downy mildew of cabbage and that phosphorus seems to decrease it. The one year's results do not permit the drawing of conclusions.

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RELATION OF ENVIRONMENTAL FACTORS TO GROWTH AND PATHOGENICITY OF PYTHIUM ISOLATED FROM ROOTS OF SUGAR CANE

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The disease of sugar cane usually referred to as root rot and growth failure is of great economic importance in most sugar-producing countries. The disease retards the growth of the cane, following a deterioration of the roots. Investigators have attempted for many years to determine the cause of this disease, and, from time to time, various organisms or soil conditions have been stated to be important factors. The prevailing opinion as has been brought out by Edgerton, Tims, and Mills (5), is that a number of factors are involved.

One of the factors generally recognized to be important in the root-rot problem is the root decay caused by species of Pythium or Pythium-like fungi. Carpenter (2), Bourne (1), and Edgerton and his coworkers (4, 5) have all shown that certain species of Pythium attack the roots and produce a soft, flabby rot of the root tips. The percentage of roots thus affected under field conditions is frequently very high. At the same time, the investigations in Louisiana (5) have shown that the species of Pythium capable of rotting cane roots are universally present in fields of healthy cane as well as in those suffering from root rot. From this, it seems possible that the severity of Pythium injury depends more or less on environmental conditions.

To obtain some information on the importance of various environmental factors in the root-rot problem a series of experiments have been conducted to determine the influence of moisture, temperature and hydrogen-ion concentration on the growth of Pythiums in culture and on the development of Pythium root rot of corn.

In the infection experiments, corn was used instead of sugar cane. Earlier investigations at the Louisiana Agricultural Experiment Station had shown the behavior of the parasitic Pythiums almost the same on both. Sugar cane is propagated vegetatively, and, because the buds usually germinate very unevenly and the young plants grow slowly during the winter months, it cannot be so readily employed in infection experiments as corn, nor does it yield such reliable results.

TEMPERATURE STUDIES OF PYTHIUM IN CULTURE

The effect of temperature on the rate of growth of sugar cane Pythiums was obtained with eight different cultures. Four of these (931, 1432, A,

and B) were parasitic forms isolated from cane roots in Louisiana. These produced the lobed, enlarged hyphae characteristic of the species known to be the most important form found on cane. A fifth culture (1285), which came from Hawaii, was obtained from cane roots affected with the Lahaina disease. This culture also produced enlarged hyphae. Two other cultures (1436 and C), also isolated from cane roots in Louisiana, were only slightly parasitic. The eighth culture (D), although isolated from decaying cane roots, was of a nonparasitic species.

To obtain the effect of temperature on these organisms transfers were made from the margins of rapidly growing cultures to petri dishes containing Difco dextrose agar. The plates were placed in constant-temperature incubators which had a temperature range of 15° to 36° C. The increase in diameter of each colony was measured at 12-hour intervals and the average growth in three plates obtained. The results are given in table 1.

TABLE 1.—*The influence of temperature on the rate of growth of sugar-cane Pythiums on dextrose agar*

Temperature, degrees C.	Culture number or designation							
	931	1432	A	B	1285	C	1436	D
	Diameter increase of colonies in 12 hours (cm.)							
15	1.3	1.1	1.0	0.9	1.1	0.9	0.9	0.7
20	2.1	2.0	1.8	1.7	1.9	1.9	2.1	0.8
25	2.5	2.6	2.6	2.5	2.6	2.5	3.2	1.0
30	2.8	2.9	2.9	2.9	2.9	3.2	4.0	1.1
36	1.4	1.7	1.7	1.4	2.0	2.5	3.2	0.5

As shown in the table, the growth of all the parasitic cultures was nearly the same. The rate of growth increased with the temperature up to 30° C. and fell off markedly at 36° C. Thirty degrees was also the most favorable temperature for the mildly parasitic forms, but these made a fairly good growth at 36° C. The last two cultures seemed to be somewhat different, 1436 growing considerably faster than C at all except the lowest temperature. The nonparasitic form grew slowly at all temperatures.

Although the differences in the rate of growth of the parasitic cultures were slight, the results indicated that at lower temperatures 931 grew faster than 1432, while at higher temperatures the reverse was true. Because of this, these two cultures were studied at narrow temperature ranges. From this study it was found that, at 25° C., the rate of growth was approximately the same for both. At temperatures below 25°, 931 grew somewhat faster

than 1432, while at temperatures above 25° it grew more slowly. These results are of interest because 931 had been growing in culture for three years and 1432 only one year. In this time 931 had become decidedly less parasitic than it was at first.

RELATION OF H-ION CONCENTRATION TO GROWTH

Early in the investigations on the root-rot problem it was observed that parasitic Pythiums were rarely obtained when diseased roots were plated on acidified media but were readily obtained on nonacidified media. It was thought desirable therefore to obtain more information concerning the influence of H-ion concentration on the growth of Pythium.

A number of nutrient solutions were used in preliminary trials. The one judged most suitable consisted of 20 grams of dextrose, 3 grams of beef extract, and 5 grams of peptone made up to 1000 cc. with distilled water.

The usual technique for this type of work was employed. The beef extract, peptone, and sugar were obtained from the Digestive Ferments Company. Pyrex glassware and water distilled over block tin were used. The H-ion determinations were made colorimetrically with LaMotte indicators. Because sugars react with acids and alkalies when heated under pressure, these were sterilized separately. The nutrient solution was made up to 600 cc. instead of a liter. Flasks of 250 cc. capacity were used, 60 cc. of the concentrated solution being placed in each flask. Sufficient distilled water was then added so that, when the required amount of tenth-normal acid or alkali was added after sterilization, the total volume in each flask was 100 cc. These flasks were inoculated and the amount of growth was determined at weekly intervals by collecting the mycelium on a weighed filter paper dried to constant weight in an oven at 100° C. The paper and mycelium were weighed after these had been similarly dried.

Twenty-four flasks of medium were prepared for each H-ion concentration to be tested. Eighteen flasks were inoculated with Pythium 1432 by transferring a small portion of the medium and mycelium from the margin of a three-day-old dextrose-agar culture to each flask. Six flasks were kept as controls. It had been planned to determine the pH and amount of fungous growth in three inoculated and one control flask each week for a period of six weeks, but contaminations limited the experiment to five weeks. The results of the tests are given in table 2.

The results in the table indicate that a neutral or slightly alkaline medium is most favorable for growth of Pythium. The fungus did not grow in solutions of initial pH 4.3 or 4.6. Growth was retarded at first in those flasks having an initial pH of 5.3, but by the end of three weeks it was as great as in solutions of a more favorable H-ion concentration. Best

TABLE 2.—*Relation of hydrogen-ion concentration to growth of sugar-cane Pythiums on acid and alkaline media for different lengths of time from date of inoculation*

N/10 HCl or NaOH in 100 cc.	pH after 72 hours	*1 week* pH		Weight mycelium in mg.	*2 weeks* pH		Weight mycelium in mg.	*3 weeks* pH		Weight mycelium in mg.	*4 weeks* pH		Weight mycelium in mg.	*5 weeks* pH		Weight mycelium in mg.
		Check	Pythium		Check	Pythium		Check	Pythium		Check	Pythium		Check	Pythium	
7.5 cc. HCl	4.3	4.3	4.2	0	4.2	4.2	0	4.2	4.3	0	4.2	4.2	0	4.3	4.3	0
5.0 cc. HCl	4.6	4.6	4.6	0	4.6	4.6	0	4.6	4.6	0	4.6	4.6	0	4.6	4.7	0
2.5 cc. HCl	5.3	5.3	5.6	54	5.2	5.8	338	5.3	6.0	598	5.2	6.7	628	5.2	7.9	532
Control	6.0	6.0	6.0	160	6.0	6.0	484	6.0	6.2	571	6.0	6.9	581	5.8	8.2	500
10 cc. NaOH	8.3	8.2	6.6	238	8.3	6.2	518	8.2	6.6	603	8.2	7.8	533	8.1	8.2	487
20 cc. NaOH	9.2	8.7	7.8	90	8.8	6.4	428	8.7	6.0	518	8.7	7.6	517	8.6	8.3	445
30 cc. NaOH	9.6[a]	9.0	8.6	31	9.1	7.2	283	9.0	6.4	404	9.0	6.5	543	8.8	7.9	486
40 cc. NaOH	9.6[a]	9.6[a]	9.6[a]	0	9.2	9.2	0	9.2	9.2	0	9.2	9.2	0	9.1	9.1	0

[a] Higher than 9.6 not determined for lack of proper indicators.

growth was obtained in the solution having an initial pH of 8.3, but growth in the solution to which no acid or alkali had been added was only slightly slower. The most alkaline media were beyond the range of the indicators, but growth was greatly retarded or stopped at a pH greater than 9.6.

The metabolic products of fungous growth changed the pH of the media towards 6.0 regardless of the initial H-ion concentration. This tendency to change the pH towards 6.0 continued as long as the mycelial felt increased in weight. After maximum growth had been reached, the solutions uniformly became alkaline, as shown in the tests made after four and five weeks.

THE INFLUENCE OF TEMPERATURE ON INFECTION

In order to determine the effect of temperature on the rotting of roots by Pythium, corn was grown in No. 2 metal cans in temperature-controlled incubators. The cans were filled with soil and then sterilized in the autoclave for two hours at 15 pounds pressure. Before planting, the soil was inoculated with pure cultures of Pythium, strains 931 and 1432. A single three-day-old petri-dish culture of the fungus on bean-pod agar served as inoculum for each can, and a similar amount of sterile bean-pod agar was placed in each control can. Five germinating seedlings of the variety Calhoun Red Cob were planted in each can. Three cans of each Pythium and the same number of checks were grown at each temperature. The soil was maintained at 60 per cent of its water-holding capacity by weighing daily. The plants were allowed to grow and the height was obtained by measuring to the end of the longest leaf. The amount of rot present on the roots was also observed. The results of this test are given in table 3.

TABLE 3.—*Influence of temperature on growth of corn in Pythium-infested soil in temperature incubators*

Temperature degrees C.	Period of growth	Average length of tops in cm.			Percentage decrease	
		Check	Pythium 931	Pythium 1432	Pythium 931	Pythium 1432
35	5	24.2	25.8	22.2	6[a]	8
30	5	30.0	23.5	19.0	22	37
26	5	26.8	15.4	12.0	43	55
23	5	17.4	12.2	10.8	30	38
20	10	30.0	19.0	19.6	37	35
15	20	19.5	10.1	9.3	48	52

[a] = Increase.

Both strains of Pythium were severely parasitic at the intermediate and lower temperatures, but 931, which had been in culture three years, ap-

peared to be less parasitic than 1432, which had been in culture only one year. A temperature of 35° C. was too high for the best growth of corn, but at this temperature there was very little rotting of the roots. At 30° C. the corn grew best, although there was considerable rotting. As the temperature was lowered, Pythium injury to the roots became more severe, and at 15° C. even the main roots were rotted off nearly to the seed. These results agree closely with those of Johann et al. (6), who found that *Pythium arrhenomanes* Drechs., which causes a root rot of corn in Wisconsin and is very similar to the cane Pythium (3), was more pathogenic at low than at high temperatures and in wet than in dry soils.

Another form of Pythium injury was noted at the low temperatures. In a number of cases there was a rot of the mesocotyl and the stem above the seed, and the Pythium mycelium was found in the decayed areas. This type of injury was not found on corn grown at temperatures above 23° C. An occasional plant was found partly rotted at 23° C., but at 20 and 15° C., this type of rotting was quite prevalent.

INFLUENCE OF SOIL MOISTURE AND TEMPERATURE ON PYTHIUM INFECTION

To determine the effect of soil moisture on Pythium infection on corn roots, a series of tests was run in the greenhouse in the spring of 1928. Cans of No. 2 size were filled with a good mixed soil and, after sterilization in the autoclave at 15 pounds pressure for two hours, each was inoculated with strain 931, a three-day-old petri-dish culture of a parasitic sugar-cane Pythium. Each can was then planted with surface-sterilized corn kernels of the variety Calhoun Red Cob. Water was then added so that the moisture content of the soil varied from 20 per cent to 100 per cent of the maximum water-holding capacity of the soil. Quadruplications of each variant were used. In order to determine the effect of temperature variations also, the test was run every month from March to June. The soil temperatures in the greenhouse during the test periods were as follows:

	Minimum	Maximum	Mean
	°F.	°F.	°F.
March	56	67	60
April	51	72	63
May	64	79	71
June	75	88	82

The germination of the seed and the height of the plants were used as criteria of Pythium injury. The results of these tests are given in tables 4 and 5.

TABLE 4.—*Influence of soil moisture and temperature on the growth of corn in Pythium-infested soil*

Percentage of water-holding capacity of the soil	Length of tops in cm.							
	March 1–11		April 2–21		May 2–21		June 8–19	
	Check	Pythium	Check	Pythium	Check	Pythium	Check	Pythium
20	5.6	4.3	3.0	3.0	8.5	7.5	4.6	3.3
30	12.2	8.1	10.9	11.9	18.7	16.9	8.3	6.3
40	14.9	9.8	16.5	12.7	26.4	22.3	16.2	13.1
50	17.2	10.8	20.9	12.1	34.0	22.2	23.1	19.9
60	17.8	10.3	25.0	11.6	38.4	19.7	26.7	18.5
70	18.3	8.1	22.6	9.1	36.4	16.3	28.3	18.4
80	18.9	10.0	23.4	8.5	38.8	11.5	29.6	17.2
90	14.5	0.0	20.6	0.0	0.0	0.0	28.6	15.4
100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 5.—*Influence of soil moisture and temperature on the germination of corn in Pythium-infested soil*

Percentage of water-holding capacity of the soil	Percentage of germination							
	March 1–11		April 2–21		May 2–21		June 8-12	
	Check	Pythium	Check	Pythium	Check	Pythium	Check	Pythium
20	12.5	7.5	2.5	10.0	77.5	75.0	12.5	10.0
30	95.0	92.5	87.5	77.5	97.5	90.0	27.5	45.0
40	100.0	95.0	97.5	90.0	90.0	95.0	92.5	80.0
50	97.5	80.0	97.5	85.0	92.5	90.0	97.5	95.0
60	87.5	55.0	80.0	57.5	85.0	77.5	90.0	95.0
70	77.5	37.5	62.5	32.5	80.0	45.0	97.5	95.0
80	70.0	15.0	30.0	20.0	12.5	17.5	85.0	72.5
90	35.0	0.0	12.5	0.0	0.0	0.0	67.5	40.5
100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The data in table 4 show that reduction in growth due to Pythium was most pronounced during the cooler months and in the wetter soils. Soil maintained at 20 and 30 per cent of its water-holding capacity was too dry for good growth of corn, but in this dry soil there was little Pythium injury. As the soil moisture was increased, the difference between the corn growing in the inoculated and in the noninoculated soils became greater, but the size of the plants in the Pythium soil increased with the increase in moisture content until the 50 per-cent series was reached. In Pythium soil kept wetter than 50 per cent of its water-holding capacity, the corn plants decreased in size with increase in soil moisture; whereas, in the noninoculated

soil, the plants increased in size with the soil moisture until the 80 per-cent series was reached. Injury due to Pythium was also more pronounced during the cool than during the warm months. In March, April, and May, the corn growing in the Pythium-infested soil kept at 50 per cent of its water-holding capacity was only about 60 per cent as tall as the check, while, in June, it was 85 per cent. In figures 1 and 2 are shown plants from the March and May series.

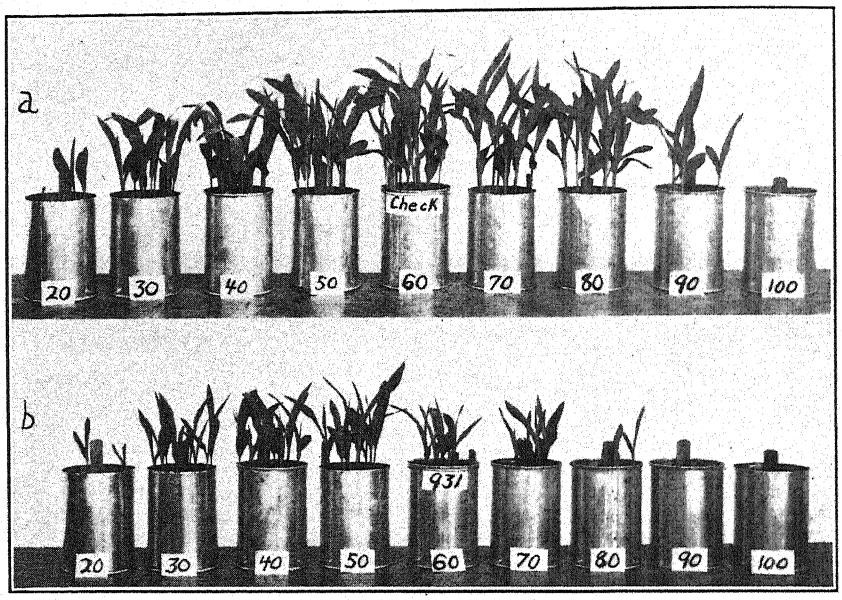


FIG. 1. Relation of soil moisture to Pythium injury. (a) Corn plants grown in sterile soil with the moisture ranging from 20 per cent to 100 per cent of the water-holding capacity of the soil. (b) Similar to the above, except the soil was reinoculated with Pythium. Growth from March 1 to March 10, 1928.

The germination results are quite regular except at the extreme moisture variations where the soil was too dry or too wet. The inoculation of the soil with Pythium did not materially affect the germination of corn in soil kept at 30 or 40 per cent of its water-holding capacity. In the series conducted during the cool months of March and April there was a noticeable reduction in germination at 50 per cent in the Pythium-infested soil; in the May series this reduction was evident at 60 per cent; while, in June, the 80 per-cent Pythium-infested soil was the first to show a significant reduction in germination. These tests indicate that both the growth and germination of corn in Pythium-infested soil are closely correlated with soil moisture and temperature.

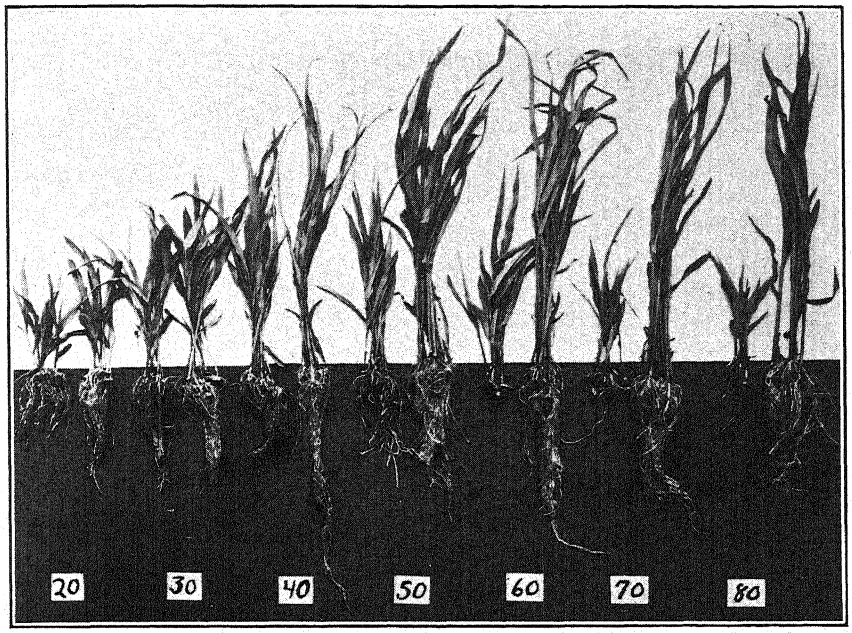


FIG. 2. Relation of soil moisture to Pythium injury. Corn plants grown in sterile soil and in sterile soil reinoculated with a pure culture of Pythium. Of each group of two plants the one on the left was from the inoculated soil. Moisture content of soil varied from 20 per cent to 80 per cent of the water-holding capacity of the soil. Growth from May 2 to May 21, 1928.

DISCUSSION

These studies were undertaken in order to obtain additional information regarding the environmental factors which influence the severity of the decay of cane roots caused by species of Pythium. The results obtained have given some interesting information in regard to the effect of temperature, soil moisture, and soil acidity.

It is known that root rot of sugar cane is more severe in poorly drained fields and in seasons of excessive rainfall (4) and exceptionally low temperature in January, February, and March. This is explained to a certain extent by the results given in this paper. Although the growth rate of Pythium increases with the temperature up to 30° C., the effect of the fungus on growing plants is more severe at lower temperatures. Only young terminal portions of the roots are subject to attack and it is possible that the cool weather holds the roots in a susceptible condition longer than warm weather.

The results obtained in the soil-moisture studies agree closely with conditions in the field. The effect of the fungus was severe only in soils with a

water content greater than 50 per cent of the moisture-holding capacity. This emphasizes the importance of the best drainage possible, especially during winter and spring, when the cane grows very slowly.

The hydrogen-ion studies show that the Pythium fungi can grow readily in any of the Louisiana soils used for cane culture. The results indicate that the fungi grow well from pH 5.6 to 9.2, and most of the cane soils are in the neighborhood of the neutral mark.

SUMMARY

1. The growth rate of strongly parasitic, mildly parasitic, and nonparasitic cultures of Pythium, isolated from the roots of sugar cane, increased with the temperature up to 30° C. The rate of growth of the strongly parasitic cultures fell off sharply at 36° C.

2. A parasitic Pythium grew well in solutions of pH 5.3 to 9.2. It did not grow at pH 4.6.

3. Pythium injury to germination and growth of corn decreased with rise in temperature. At 35° C. Pythium did not injure corn. At 30° C. there was an appreciable amount of injury and, as the temperature was lowered, injury became more severe.

4. Pythium injury to germination and growth of corn increased with the water content of the soil but was less severe in wet soils in warm than in cold weather.

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THE CHERRY LEAF-SPOT FUNGUS, MYCOSPHAERELLA CERASELLA ADERH., ITS MORPHOLOGY AND LIFE HISTORY

W. A. JENKINS[1]

Toward the end of summer, cultivated cherries in North Carolina become prematurely defoliated by the conidial form of a fungus commonly referred to as *Cercospora cerasella* Sacc. This organism appears to be of rather common occurrence on the several species of cherries but is of little economic importance anywhere. It has been reported to be present on *Prunus avium* L. in Georgia and Alabama, on *P. demissa* D. Dietr. and *P. virginiana* L. in Kansas, and on *P. cerasus* L., both sweet and sour varieties, in Alabama, Mississippi, and Florida (2). Saccardo (6) records its occurrence on cherries in France and Italy and Aderhold (1), in Germany.

This leaf-spot disease can be distinguished readily from such diseases as shot hole, rust, and mildew, which are more or less generally prevalent throughout the State. Its lesions are at first pin-point-like, circular, yellow spots present on either leaf surface. As they enlarge to their mature diameter of a millimeter or more, the central portion becomes reddish brown with a border of lighter color. The necrotic tissues gradually become dry and brittle and when the lesions are abundant they may coalesce to form large, irregular, dead areas. In the case of severe infections rather serious defoliation results. The reserve food stored for the succeeding season is in consequence diminished. Either or both leaf surfaces of mature lesions are covered with dark fascicles of conidiophores and conidia.

Apparently the only published account of the disease and of the life history of the pathogene is that of Aderhold (1) in 1900. Beginning in 1894, he observed the disease throughout several seasons and noted that during winter an ascigerous stage appeared on fallen leaves near old Cercospora lesions. He isolated the organism from ascospores and obtained in these cultures conidia of a Cercospora together with structures which he interpreted to be perithecial initials. He also observed that fallen diseased leaves, which remained out of doors during winter and were then brought into the laboratory during April and May and placed in moist chambers, developed conidiophores and conidia of the Cercospora type. On the basis of these findings he concluded that the ascigerous stage, which he designated *Mycosphaerella cerasella* n. sp., is the perfect stage of *Cercospora cerasella* Sacc.

The present study, which extends over the past two years, deals with the development of the fungus in leaves, in both natural and artificial

[1] Acknowledgment is made of the counsel and help of F. A. Wolf, under whose direction this study was conducted.

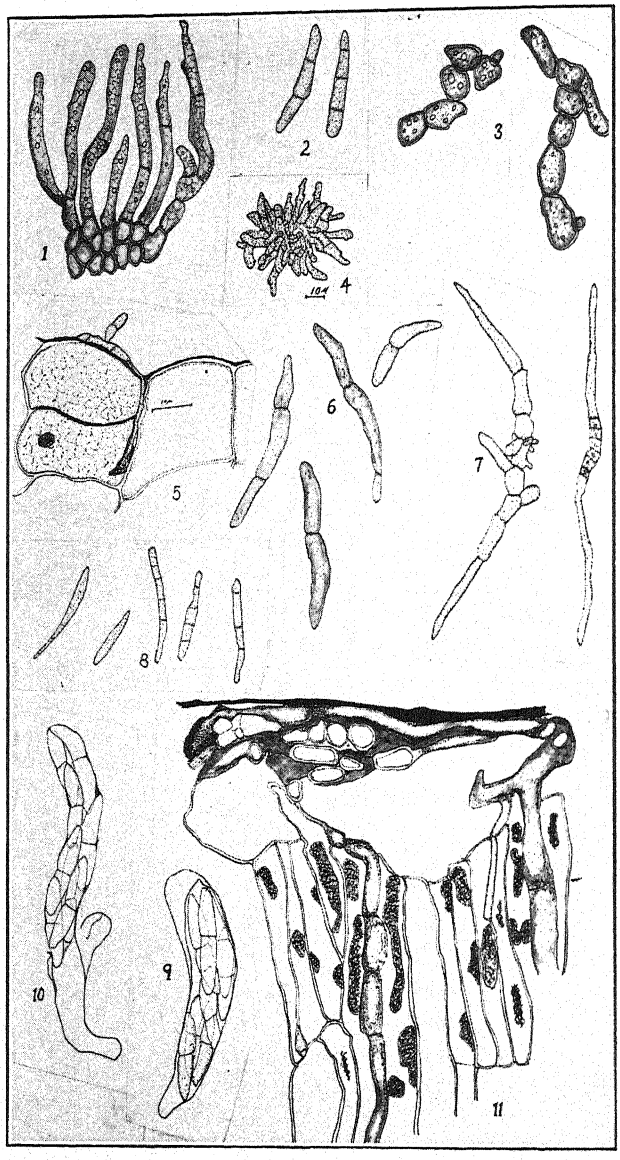


FIG. 1. 1. Conidiophores arising from a stromatic base; 2. Conidia developed in culture; 3. Chlamydospore-like cells of the type commonly present in cultures; 4. Conidiophore fascicle in surface view; 5. Penetration of germ tube from conidium, in transverse section of leaf; 6. Germination of ascospores of *Mycosphaerella cerasella* Aderh.; 7. Germination of conidia; 8. Conidia from leaf lesions; 9. Mature ascus; 10. Ascus which has elongated preparatory to ascospore discharge; 11. Relationship of pathogene and suscept shown in transverse section of lesion. 1 and 11 drawn to scale shown near figure 2, 13; 2, 3, 5, 6 and 8–10 to scale near 5; 4 and 7 to scale near 4.

inoculations, and in artificial cultures, with special emphasis on the morphology and genetic relation of the several stages. It therefore supplements the previous study by Aderhold (1).

Infected leaves were collected at frequent intervals throughout the year. They were then fixed in Bouin's fluid, appropriately sectioned, and stained with Haidenhain's iron alum haematoxylin.

The germ tubes from conidia sown on the surface of living leaves were found entering the leaves by direct penetration (Fig. 1, 5). It would appear that they could enter also through the stomata which occur on the dorsal leaf surface only. The mycelium is at first confined to the intercelluar spaces, but upon death of the cells it enters them (Fig. 1, 11). There is no evidence that the pathogene is able to kill cells in advance or actual extension of the mycelium.

CONIDIAL STAGE

By the time that well-defined lesions are evident the conidial stromata have begun to develop subcuticularly or within substomatal cavities. As the result of continued growth compact masses of stromatic tissue are developed, which serve as the bases of the conidiophores. (Fig. 1, 1.) These conidiophores extend outward and project from the leaf surface either through fissures in the cuticle or through the stomatal orifices. Each conidiophore fascicle consists of a small group of spreading elements (Fig. 1, 4), hyaline at first and brown at maturity, from the tips of which the conidia are abjointed. As the conidia mature they, too, gradually become brown. After a conidium is abstricted and falls away, leaving a scar, the conidiophore continues to grow beyond this scar. By the repetition of of this process the conidiophore becomes geniculate and flexuose. The conidia are obclavate, bent, pale brown, septate, guttulate, and measure 40–60 by 3–4 μ (Fig. 1, 8).

SPERMOGONIA

Spermogonia are formed on both leaf surfaces of fallen leaves throughout the fall and early winter. They originate either within old conidial stromata or within separate stromata that develop after the death of the leaves. Mature spermogonia were first noted in sections of material fixed during the latter part of October and spermatial production ceased by the latter part of December.

In studying the course of spermogonial development use was made of sections of infected leaves and of spermogonia produced in artificial culture. They arise meristogenously (Fig. 2, 16 and 17) by the branching of a hypha and the fusion of these branches. Then, by a rapid division

of cells, a spherical stromatic mass is formed (Fig. 2, 18). The cells at the center of this mass, which are to become the spermatium mother cells, become larger and stain deeper than those of the periphery. The nucleus of each mother cell then divides and 3–4 spermatia are formed endogenously (Fig. 2, 19). The spermatia are liberated through a sterigma-like process, figure 2, 19, after which the walls of the old mother cells disintegrate into a mucilaginous matrix which invests the spermatia. This process continues centrifugally so that a mature spermogonium (Fig. 2, 20) consists of a globose cavity, filled with spermatia held together in a mucilaginous matrix of degenerating mother-cell walls. Outside of this is a layer of fertile cells densely filled with protoplasm and then the spermogonium wall, itself, consisting of two or three cell layers of brown, thick-wall elements. Mature spermogonia measure 36–90 by 25–73 μ and spermatia 2.5–2.7 by 0.9–1.0 μ.

This method of spermatial formation appears to be quite similar to that described by Higgins for *Mycosphaerella bolleana* (4) on fig and *M. personata* (5) on grape. Whether or not it is of any phylogenetic significance is conjectural, especially since no Ascomycetes are known to give rise to conidia in this manner.

PERITHECIA

Perithecia form most abundantly on the surface of the leaf in contact with the soil. They begin their formation about the same time as do the spermogonial primordia but do not become sufficiently differentiated to be recognized as perithecial primordia until late November, about a month after mature spermogonia are first noted. At this time the developing stromata have differentiated into a peripheral portion with brown, thick-wall cells and a medullary portion with thin-wall cells, rich in protoplasm. In this central portion there is a coiled, coenocytic carpogonium which arises near the base of the stroma and projects, by means of a trichogyne, nearly or entirely through the cells at the apex of the stroma (Fig. 2, 12). Apparently, each stroma normally has a single carpogonium, although two perfectly formed ones were observed in one case.

As growth continues, more deep-staining cells form around the carpogonium. Apparently these cells function as a source of nourishment for the developing carpogonium and later, those which remain, as a reserve for the rapidly growing asci. Meanwhile the trichogyne degenerates, beginning at the tip, and the basal portion becomes the ascogonium. By December 12, ascogonia of the type shown in figure 2, 13, had formed. Various stages in this process of differentiation could be found in collections made during the period extending from late November to late December. Each ascogonium remains coenocytic but the nuclei become as-

sociated in pairs, presumably by nuclear migration. At least there is no evidence that one of the members of each pair of nuclei comes from an antheridium or any structure remotely resembling an antheridium as described in *Venturia inaequalis* (Cke.) Wint. (3). During the period which follows, the ascogonia enlarge and the nuclei multiply, apparently by conjugate division. Further, there is no evidence of fusion of these paired nuclei within the ascogonia. Instead, they remain associated with no actual fusion except within the young asci which may first appear several weeks later.

Various stages in the development of asci were observed in collections made in the period extending from the latter part of December to the middle of March. Asci arise as branches extending outward from the ascogonium. There is no evidence of crozier formation. Instead, each ascus is a separate branch originating directly from the ascogonium so that each ascogenous hypha is actually a young ascus (Fig. 2, 15). This is entirely like the condition described in *Mycosphaerella bolleana* (4).

Not all asci arise at the same time nor do they develop at the same rate and, consequently, do not mature at one and the same time. Within a single perithecium various stages in development of asci can be noted, ranging from very young ones to mature ones. It would therefore be anticipated that there would be an extended period of ascospore discharge from any individual perithecium. In fact, the period of spore expulsion has been found to extend from the middle of March throughout the entire summer and into early September. This sustained period can be accounted for in part, by the lack of uniformity in development of asci within individual perithecia. The further observation that, in March, some perithecia were in the same state of maturity as were others in the previous December may also account, in part, for this sustained period of ascospore discharge.

A pair of nuclei migrate from the ascogonium into each young ascus and there fuse, thus constituting the primary fusion nucleus. This is followed by three succesive nuclear divisions, so that the normal number of eight free nuclei is formed. The ascospores are next delimited uniseriately and at first each is continuous and uninucleate. Then follows a division of each ascospore nucleus and the septation of each ascospore into two unequal cells, the upper of which is the larger. Meanwhile, they have become oriented in distichate fashion. Mature ascospores range in size from 13–17 by 3–4 μ.

The asci are clavate-cylindrical with a thickened, slightly excentric papilla. They measure 40–60 by 8–10 μ (Fig. 1, 9). The walls are composed of two membranes the outer of which ruptures and is thrust aside as the inner membrane elongates to about twice its normal length preparatory to expulsion of the ascospores (Fig. 1, 10). By this mechanism the

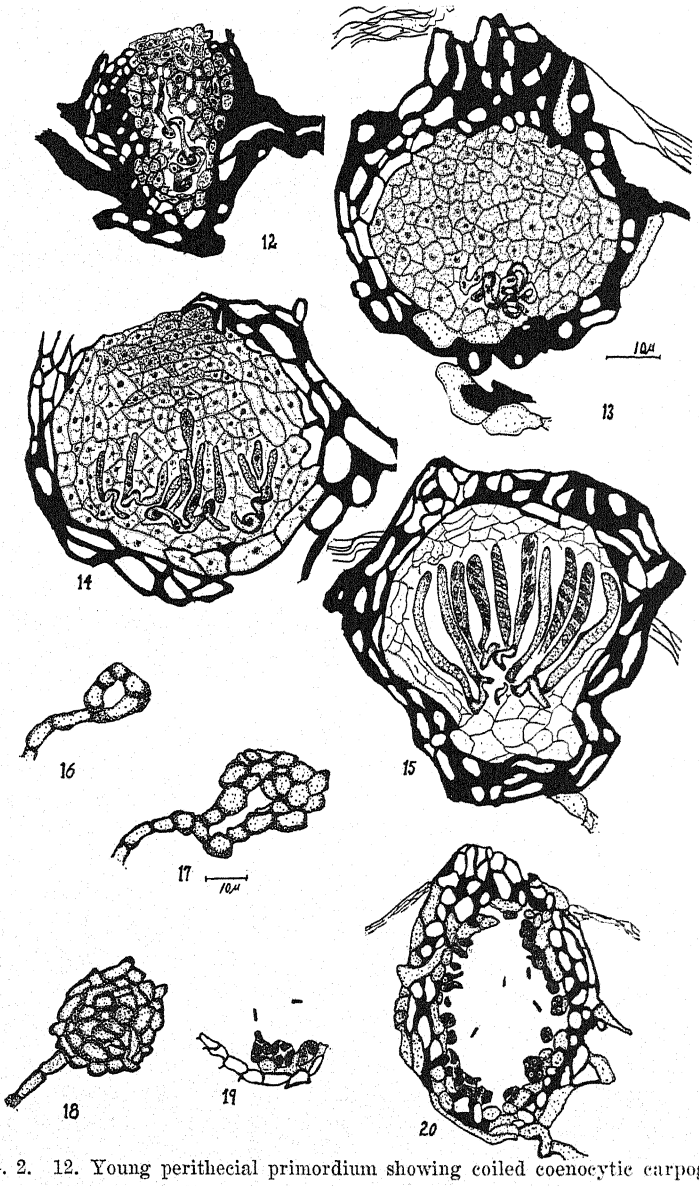


FIG. 2. 12. Young perithecial primordium showing coiled coenocytic carpogonium; 13. Later stage in perithecial formation showing ascogonium with paired nuclei; 14. Young asci arising from an ascogonium; 15. Perithecia with well-developed asci just prior to the formation of the ostiolum; 16, 17, and 18. Successive stages in spermogonial formation; 19. Portion of spermogonial wall showing spermatium mother cells and spermatia; 20. Nearly mature spermogonium in detail with only a few spermatia which normally fill the central cavity. 12–15, drawn to scale shown near 13; 16–20 to scale near 17.

asci extend into the ostiolum and discharge their spores to the exterior. They then collapse and thus afford opportunity for spore discharge by other asci within the same perithecium.

The stromata from which perithecia arise have in some cases been found to be the stromata which had previously borne conidiophores, in others, separate stromata, but they are always located wholly within the leaf tissues. As the perithecia approach maturity, during ascus formation, they either rupture the epidermis or distend it and appear, under the hand lens, as dense clusters of black projections. In section these show that each perithecium remains closed with no evidence of an ostiolum until the ascospores are essentially mature. Each perithecium is capped by a tissue of brown, thick-walled cells (Fig. 2, 15). Just beneath this cap and above the asci is a tissue of thin-walled, fragile cells similar to those which disintegrate as the asci enlarge. It would appear that pressure exerted by the developing asci must push apart the lateral walls of the perithecium and thus tend to form a pore or ostiolum at the top. At the same time the thin-walled tissue above the asci proliferates and, in so doing, forms a lining for the ostiolum. This type of development of ostiola resembles that recently described by Higgins (5) for *Mycosphaerella personata*.

GENETIC RELATIONSHIP OF THE SPORE FORMS

The genetic connection of conidia, spermogonia, and perithecia of the cherry leaf-spot fungus rests upon the following evidence:

(1) Spermogonial and perithecial primordia have been observed to arise, as has already been stated, in the bases of conidial stromata within fallen leaves. Moreover, a characteristic mycelium consisting of thick-walled, large, brown cells ramifies throughout the leaf tissues, extending from the conidiophore bases and also from spermogonia and perithecia and could readily be traced from any one of these structures to any other.

(2) Typical Cercospora conidia have been developed in artificial cultures made from ascospores of this leaf-spot organism. Curiously, when conidia or ascospores were planted on such media as potato agar, corn-meal agar, bean agar, dextrose agar, synthetic nutrient agar, or cherry-leaf-decoction agar, to each of which fragments of diseased leaves had been added as an additional source of nutriment, no fruiting was obtained, although the fungus made very satisfactory vegetative growth. If, however, tubes of these media, on which the organism had been growing for a time, were resterilized and reinoculated, conidia were produced (Fig. 1, 2), together with spermogonia bearing spermatia and stromatic bodies which were apparently perithecial primodia, when cultures made from ascospores were the source of inoculum. When the inoculum came from cultures

made from conidia the results were identical, except that conidia were not observed to have been developed. Neither asci nor ascospores, however, were ever noted in these stromatic bodies.

All efforts to germinate spermatia both from leaves and from culture failed. This failure accords with the observed behavior of spermatia of other Ascomycetes.

(3) Small cherry trees, the leaves of which showed no signs of infection, were set in the greenhouse in December and inoculated on April 4 with ascospores. Infection resulted and on April 25 mature lesions had developed which bore conidia of *Cercospora cerasella*. The ascospores used in these inoculations were obtained by placing perithecia-bearing leaves beneath inverted agar plates. The spores, on expulsion, lodged on the surface of the agar above. In inoculating, blocks of agar with adhering ascospores were cut out and placed in contact with the leaves. The leaves were than wrapped for 24 to 48 hours in oiled paper to conserve moisture. Inoculated leaves, only, became infected.

An unidentified species of Mycosphaerella with somewhat smaller ascospores than the pathogene was found to be rather commonly associated with it on overwintered leaves. This unidentified organism was isolated in pure culture and compared with *M. cerasella* from which it is distinct in all vegetative characters. No conidia developed in these cultures. Moreover, no infections were secured following inoculation by the same method successfully employed with the pathogene.

(4) The development of conidial, spermogonial, and ascigerous stages by the cherry leaf-spot fungus, in sequence in the same lesion, is in accord with an increasing body of knowledge of the course of development in the life cycles of Ascomycetes.

These observations and results, when regarded as a unit, justify the conclusion that the three stages, herein described, belong to one and the same fungus, *Mycosphaerella cerasella*. Since, in this study, the complete life cycle of the cherry leaf-spot fungus is determined for the first time, a brief description of it is included.

MYCOSPHAERELLA CERASELLA ADERH.
SYN. CERCOSPORA CERASELLA SACC.

Ascigerous stage: Perithecia densely aggregated, amphigenous, innate but erumpent at maturity, globose, black, ostiolum only slightly papillate, 60–120 μ in diameter; asci clustered, bluntly clavate-cylindrical, aparaphysate, thickened by an excentric papilla at the apex, eight-spore, 40–60 by 8–10 μ; ascospores imperfectly biseriate, unequally bicellular, the upper cell somewhat larger, slightly curved, hyaline, 13–17 by 3–4 μ.

On fallen decaying leaves of *Prunus* spp., maturing during the period extending from March to September.

Spermogonial stage: Spermogonia densely scattered in and near lesions produced by the conidial stage, globular to flask-shape, black, embedded in the leaf tissue at first, later erumpent, ostiolate, 36–93 by 25–72 μ; spermatia rod-shape, 2.5–2.7 by 0.9–1.0 μ, arising endogenously, usually in fours within spermatiferous cells, and liberated by sterigma-like projections, hyaline.

On recently fallen leaves, maturing throughout the period extending from late October throughout December.

Conidial stage: Lesions circular to irregular, often confluent, varying in size, pale brown to dark brown; conidiophores amphigenous, arising from a stroma, spreading in a lax fascicle, geniculate, flexuose, brown at maturity, 30–40 μ long; conidia cylindrical to clavate, straight or curved, ochraceous to pale brown, guttulate, septate, 40–60 by 3–4 μ.

On living leaves of *Prunus* spp. in late summer.

SUMMARY

This report deals with a leaf-spot disease of cherries, caused by *Mycosphaerella cerasella,* which has three spore forms in its life cycle. Partial proof of the genetic connection of its conidial stage and its asciger-ous stage has been submitted by Aderhold in 1900. The evidence of this connection, herein submitted, is sufficient to regard it as conclusive proof. In addition there is a spermogonial stage which appears during autumn.

The morphology and course of development of each of the three spore forms are described.

The pathogene has been isolated in pure culture in which conidia and spermogonia are produced. Pathogenicity has been established by inocula-tion experiments.

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DURHAM, N. C.

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AN OFFSHOOT AND LEAFSTALK DISEASE OF DATE PALMS DUE TO DIPLODIA[1]

HOWARD S. FAWCETT[2]

In January, 1928, the attention of the writer was called to this disease on offshoots of date palms, *Phoenix dactylifera* L., by members of the staff of the United States Department of Agriculture Experiment Date Garden, Indio, California, who have given helpful cooperation throughout this investigation.

The disease had been observed by the writer on leafstalks in one date-palm garden the preceding year. It has since been found in a number of gardens throughout the Coachella Valley. One of the date growers reports having observed the offshoot form of the disease ever since date growing was established in that section. The disease appears to be most common on the Deglet Noor variety.

SYMPTOMS

In severe cases the disease results in death of offshoots either while they are still attached to the mother palm or after they have been detached and planted out. The disease sometimes also causes a premature death of leaves in older palms.

In offshoots the disease manifests itself in two distinct types; one, in which the outside leaves die first and the younger shoots and bud remain alive for some time; the other, in which the dying-back of the center of offshoot or bud precedes the death of the older leaves. It appears from the investigation and observations thus far carried on that the manner and place of infection largely determine which of these two types will occur. The infection appears to take place either at the base of the offshoot, near the place where it joins the mother palm, and probably prevents sufficient water from getting to the young leaves, or the older infection takes place in the outside leaves of the offshoot first and spreads later to the newer leaves and to the heart.

In the leaves of older palms the ventral midportion of the stalks is the part most commonly affected, showing yellowish brown streaks running upward 6 inches to 3 or 4 feet from the base (Fig. 1, A). The disease may spread laterally from one leaf base to others in close proximity. Frequently, these streaks extend upward on one of the lateral angles of the

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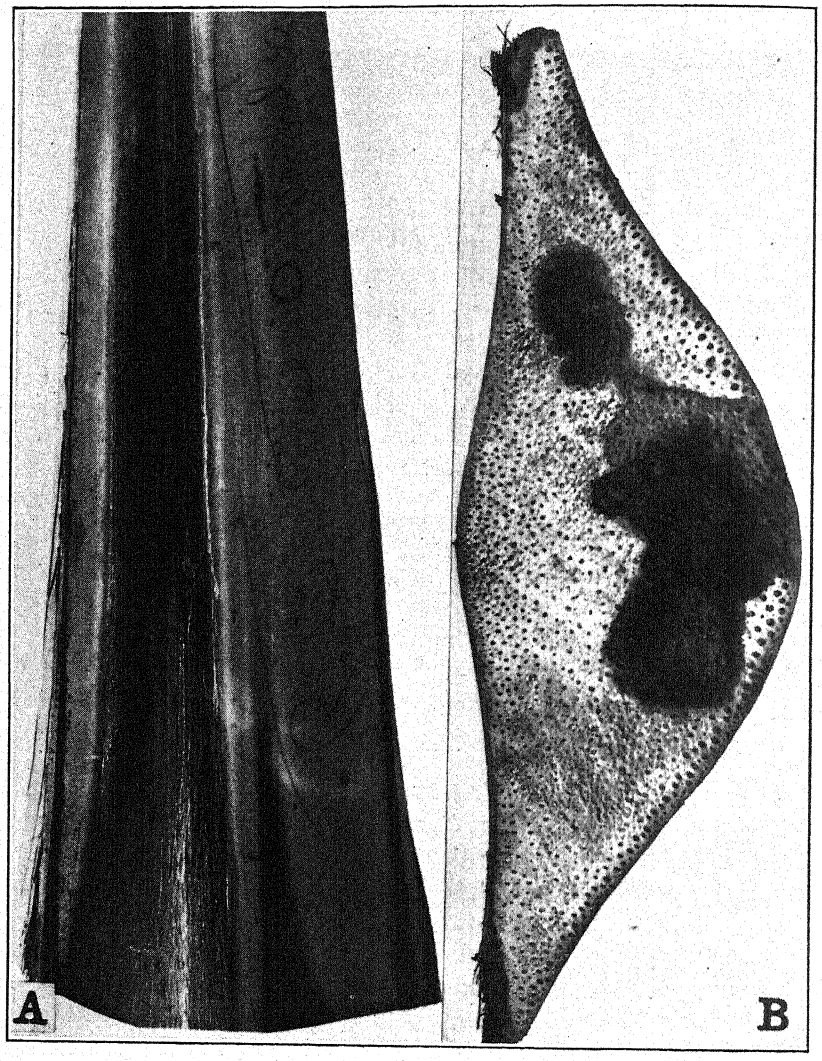


FIG. 1. A. Leafstalk of *Phoenix dactylifera* invaded by Diplodia showing discolored streak extending up from base at the midventral portion (about one-half natural size.) B. Cross section showing region of dark brown tissue in the interior of a leafstalk invaded by Diplodia. (About natural size.)

leafstalk. These streaks may sometimes extend upward long distances, while the upper portion of the leaf is still normally green and apparently unaffected. The color and size of these streaks vary according to the age of the leaves. On a leaf still green these streaks may be 3 to 4 inches wide at the base, narrowing rapidly upward to a width of half an inch or less.

The outside color near the base may be pecan brown to terra cotta. On a cross section (Fig. 1, B) the tissue may appear chestnut brown to raw umber at the center and cinnamon buff on the edges where the tissue has been more recently involved. In a large leaf these brown streaks have been observed to extend as much as 4½ feet from the base and beyond this point to extend 2½ feet farther, as a yellow streak.

ISOLATION AND INOCULATION EXPERIMENTS

Early in 1927 isolations of a Diplodia were made from the interior tissue of streaks in a leaf base and inoculations were carried out on leaf bases of a healthy palm in the same orchard in March, 1927. While infection was seen to be taking place after several months, its progress seemed to be slow and the inoculated plant was not carefully watched. Two years after the inoculation was made, not only were the infected leaves dead, but a near-by offshoot on the same palm was found badly infected. The angles of the petioles of three other leaves nearest those inoculated showed characteristic streaks extending upward from the base. The disease in this palm had all the appearances of having definitely progressed from the inoculated leaf bases. From these streaks in the leaf bases, as well as from the diseased offshoot, Diplodia was again isolated.

In January, 1928, Diplodia was again isolated from the discolored tissue of a dying offshoot in another date garden. This Diplodia culture was inoculated into the leaf bases of healthy palms in this date garden in March, 1928. In three months the largest lesion was 5 inches long and 1½ inches wide and, in seven months, it was 7 inches long and 1¾ inches wide (Fig. 2, B) and had spread inwardly almost entirely through the interior tissue of the leafstalk. This leaf was removed and Diplodia was again isolated in pure cultures from the advancing margins of the lesion. A number of other lesions, somewhat smaller in size, were produced at the same time.

A similar set of inoculation experiments was carried on in a third date garden from which Diplodia had been isolated from the leaf bases of older palms, and these gave similar results. The same fungus has been isolated from two other date orchards, making five in all. These experiments indicate that this Diplodia is the causal agent in the disease.

CONTRIBUTING FACTORS

Observations indicate that, although wounds made in pruning contribute to infection leading to the development of the disease (Fig. 2, A), such wounds are not always necessary to infection; especially, in case of offshoots. For example, in one Deglet Noor garden where the disease is doing much harm to offshoots, no pruning of leaves on either the mother trees or their

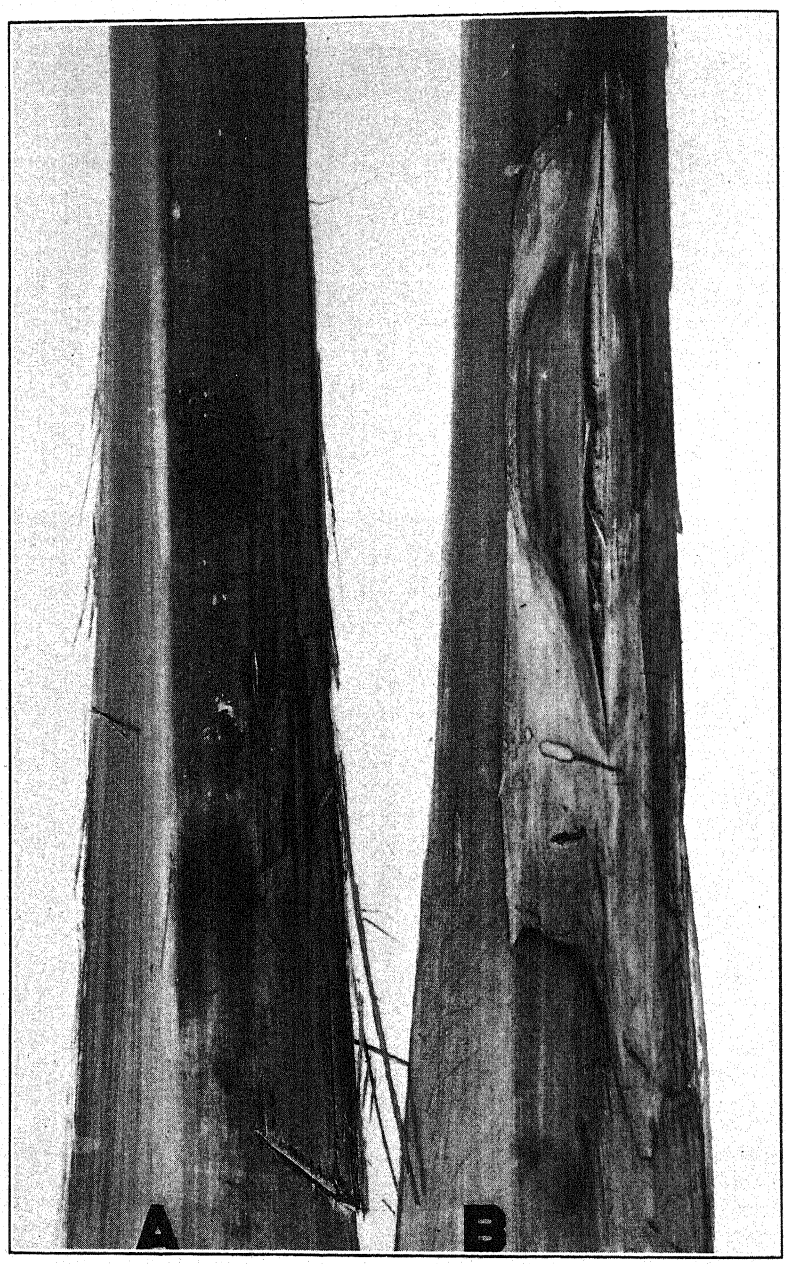


FIG. 2. Lesions on leafstalks of *Phoenix dactylifera* resulting from Diplodia infection. A. By means of an inoculation at cut occurring accidentally in pruning. B. By means of an inoculation from culture of the fungus purposely placed in a small cut near middle of present crack (crack has been elongated during development of lesion).

offshoots has ever been done except when cuts were made in removing offshoots from the mother palms for planting. These are growing in good soil and have been growing very rapidly as the result of being well fertilized and well irrigated. Many growers have assumed that dying of offshoots under such conditions was due to "crowding off" by the very rapid growth of the palms. It is possible that in some cases this rapid growth may cause strains on the union between the offshoot and mother palm, causing cracks in which infection could more readily take place. This point has not been sufficiently investigated. The type of the disease which begins first in the outer leaves either of the offshoots or of the older palms appears to be aided by wounds. Another condition that may contribute to the disease is severe defects in irrigation where main roots may be accidentally allowed to dry out and die back. Such accidental defects in irrigation practice had been reported in some of the gardens where the disease was most prevalent on the leaves of older palms. A number of other organisms also have been found which are probably secondary, but some of them may aid in increasing the severity of the disease. The principal fungi sometimes found associated with the Diplodia are *Penicillium roseum, Aspergillus niger* v. Tiegh., and species of Fusarium and Rhizopus.

CAUSAL FUNGUS

The Diplodia found in the diseased tissue is similar in size of spores to *Diplodia natalensis* Evans. It seems probable, however, that it is a different strain from the *Diplodia natalensis* that produces stem-end rot and a type of gummosis of Citrus in Florida. The fungus has not been found attacking Citrus fruits nor the bark of Citrus trees growing close to date palms in the date gardens of the Coachella Valley.

The spores are at first unicellular and hyaline like those of the form genus Macrophoma, but later, under certain conditions, they become dark and bicellular and measure 22 to 24 μ by 10 to 12 μ. The light-color spores are of about the same size. The pycnidia have not been found on the leaf bases of the older palms while they are still alive and attached to the tree under conditions in Coachella Valley, but are common on dead leaf parts at certain times. They are formed also on inside protected dead leaves of dying offshoots; and, if the stained interior tissue of the lesions on leaf bases is placed in a moist chamber, pycnidia are formed on the surfaces.

SUGGESTIONS FOR CONTROL

While results have not yet been obtained from control experiments, certain suggestions may be made. Observations strongly indicate that infection readily enters the wounds made by tools in pruning the leaves

(Fig. 2, A). It would, therefore, appear to be a proper precaution to disinfect all tools and cut places in operations where cuts have to be made. The cut surfaces made in removing offshoots from the mother plant should be disinfected and as much as possible of the dead and functionless tissue removed, disinfected, or burned. One of the date growers has reported beneficial results by such practice in planting out new offshoots in which he has removed any discolored tissue at the base and disinfected the cuts with a mixture containing 2 per cent of commercial Formalin[3] and 98 per cent water. It is also suggested that the tools be disinfected with the Formalin or some other good fungicide. As a further means of prevention, it is suggested that the palms be sprayed with an ammoniacal solution of copper carbonate. This will probably penetrate the crevices between the leaf bases better than Bordeaux mixture, since it is a clearer liquid than the latter. It is also likely to spread more readily on the waxy surface because of its more alkaline nature. Five ounces of copper carbonate, 3 pints of ammonia (26° Baumé) to 50 gallons of water may be used. The ammonia should first be diluted with 1 gallon of water. The copper carbonate is made into a paste with water and slowly added to the dilute ammonia, after which water is added to make 50 gallons.

[3] Commercial Formalin is a mixture containing 40 per cent of formaldehyde gas.

INSECTS AS POSSIBLE CARRIERS OF THE CITRUS-SCAB FUNGUS[1]

ANNA E. JENKINS

The importance of insects in the transmission of plant disease is already recognized in the literature, as shown by Rand and Pierce's (8) compilation. These authors employ the term *mechanical internal transmission* as applying to "that type of insect transmission" in which the "infective principle remains viable after passage through the alimentary tract but undergoes no appreciable multiplication or development within the insect body." In this connection it is (8, p. 205) explained that the "organisms that pass through the insect's body are in a large number of instances also carried externally. . . ." Also, it is stated (8, p. 207) that "as many as seven species of fungi causing diseases in plants have been shown capable of passing through the intestinal tract in a viable condition." Beetles, as well as flies and ants, are included among the insect carriers. None of the fungi named are Sphaceloma species nor are any of them related to this myriangioid group. Furthermore, among the beetles no Mycetaeid has been reported as a transmitting agent.

The present account contains more or less limited data relating principally to the mechanical internal transmission by insects of the Sphaceloma causing scab of Citrus, which fungus species (*Sphaceloma fawcettii* Jenkins (4) was classified as of this form genus in 1925 (4). It is thought that this data, together with the fact that the fungus concerned is a Sphaceloma (4), may serve further to explain or elucidate unsolved matters relating to the dissemination and distribution of this organism (11, p. 12). It is, of course, already recognized that this pathogene may be borne by wind (3) and by rain (11) or by these two natural agencies in combination (11). Also, there is available no little information concerning the environmental conditions under which the fungus may persist (6, 7, 11). Inasmuch as in Florida, at least, avocado, *Persea* spp., and Citrus are grown in the same nursery, or grove situations, the Sphaceloma (10) causing avocado-scab (5) would naturally occur in close proximity to the structurally similar species affecting Citrus. It would therefore seem probable that, to a greater or less extent, the data here presented might also apply to the Sphaceloma pathogenic on avocado, observed under corresponding conditions.

[1] The work reported in this paper was done in cooperation with the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and with the Department of Plant Pathology, Florida Agricultural Experiment Station.

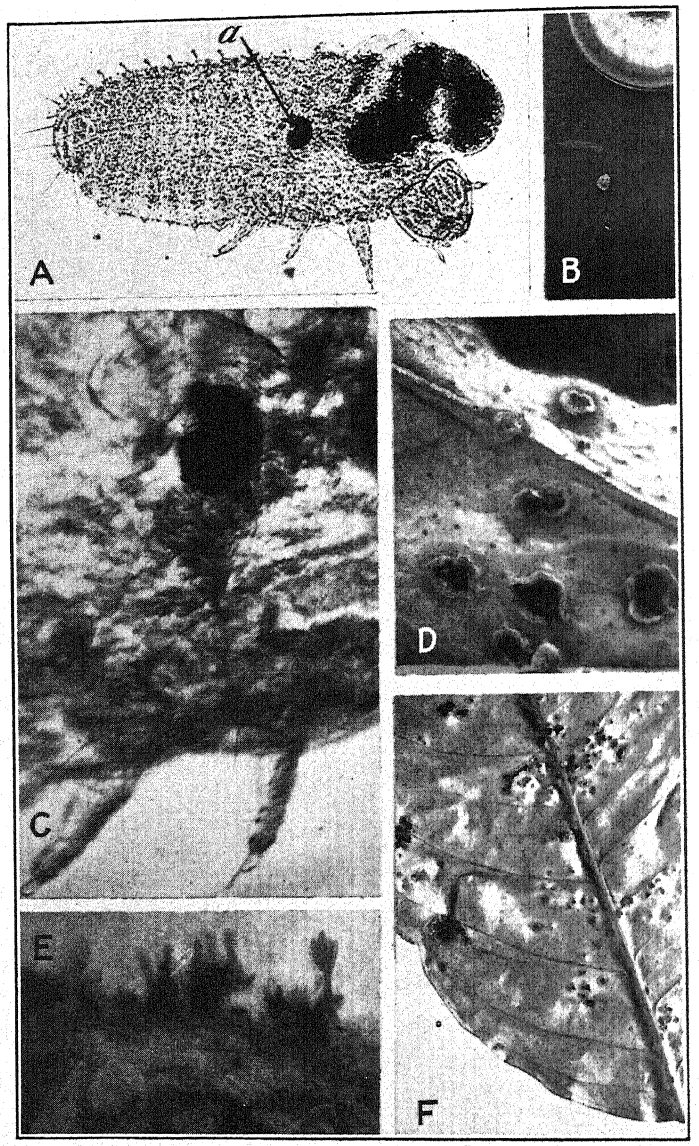


FIG. 1. A, insect discovered feeding upon conidia and conidiophores of the Sphaceloma (X approx. 85). B, part of a colony of *Cladosporium* sp. and an entire colony of the Sphaceloma, in original isolation culture (X 5/6); C, thoracic region of insect showing fructifications of the Sphaceloma scattered about the frontal end of the stomach (X approx. 300); D, enlargement of a group of scab lesions on lower part of F (X 6); E, vertical section through lesion (necrotic) showing the Sphaceloma characteristically fruiting on the lower leaf surface and, in the clear spaces in the leaf structure, intertwined hyphae of this fungus (X approx. 160). F, general appearance of Sphaceloma-covered lesions on lower surface of fresh leaf (X 5/6). B and E illustrations by J. F. Brewer; the others, by Marcel L. F. Foubert.

While examining fresh young leaves of the sour orange, *Citrus aurantium* L., quite by chance a small insect was observed in the act of feeding upon the luxuriant growth of the Sphaceloma covering the lesions or galls characteristically produced on the lower leaf surface. The leaf was one selected at random from a large assortment of about two-weeks-old leaves and stems gathered from sour-orange trees in the rutaceous collection at the Florida Agricultural Experiment Station at Gainesville. A sample of leaves from this gathering, similar to the one on which the insect was found, is shown in figure 1, D–F.

The examination, referred to above, was being made in connection with an investigation of the natural development of the Sphaceloma on fresh young growth of Citrus. This study was based not only on the rutaceous collection at Gainesville, but also on the one at the United States Department of Agriculture Citrus Diseases Field Laboratory at Orlando. The work was begun early in September and for the first three weeks was done in the field; subsequently, that is, until the end of November, it was done at Washington, D. C., where, through the kindness of Professors G. E. Weber and H. E. Stevens, and Mr. J. F. Wootten, fresh specimens of various Citrus varieties were received at desired intervals.

Only about 0.7 mm. in length, light colored and somewhat transparent in the stage observed, the insect might easily have been overlooked but for the fact that the lesions were being examined either with a hand lens or binocular microscope. From the position it assumed on the galls and the movements of its head, there seemed no question that it was feeding on the mass of dark, fuscus-color[2] conidia and conidiophores of the Sphaceloma. After busily pasturing on a given lesion for a short time, although an abundance of food still remained, it would turn quickly about, hasten to another lesion, climb up the raised margin and upon reaching the dark fungus-covered area, immediately engage in its feeding as before. This procedure had evidently been in progress for some time when the insect was first observed, for even then, because of the transparency of its rather flexible body, the stomach was visible as a nearly black, solid-appearing structure which, later examination showed, was completely filled with conidial fruiting structures of the Sphaceloma similar to those illustrated in figure 1, E.

A few moments after its feeding was thus observed, the insect was removed from the leaf, killed in alcohol, and mounted in glycerine jelly on a glass slide. Microscopic examination of the contents of the stomach was facilitated because during the process of killing and mounting, the organ became so ruptured and displaced that most of it protruded from the soft

[2] Color reading based on Ridgway (9), made by J. Marion Shull.

flexible body of the insect at a point just above the head (Fig. 1, A). Only the frontal end remained in place (Fig. 1, A, *a*; also see Fig. 1, C). The stomach was now seen to be closely packed with many hundreds of characteristically colored conidia and conidiophores of the Sphaceloma such as those illustrated in figure 1, E. It could not be determined that alimentation to this point had in any way changed the appearance of the fungus. In fact, where the conidiophores were thinly scattered, as they were about the frontal end of the stomach incident to the rupture of the canal, it was observed that they were still almost intact, some of them with the conidia apparently still attached (Fig. 1, C). Voided pellets containing this growth, and at the same time definitely known to be those of this insect, were not available, therefore, the viability of the Sphaceloma after alimentation by the insect could not be determined.

Mounted as described, the insect was identified by Dr. Adam Böving, Bureau of Entomology, United States Department of Agriculture, as the larval stage of one of the Mycetaeids, a little-known group of Coleopterous insects. The mounted specimen has been deposited in the insect collection at the U. S. National Museum.

All of the other sour-orange leaves and stems constituting the specimen on which the insect was found, as well as the specimens of sour-orange and other Citrus varieties subsequently received, were carefully inspected, either by the writer or by Dr. Böving, but no more Mycetaeids or indications of their having been present were seen, although other small insects, such as Coccids and gall mites, or their remains, were observed. These insects or remains of insects were sometimes found in close association with the lesions. A small fly also was present, moving about on most of the specimens when the packages containing them were opened. Also, while in Florida, the writer noted that medium-size ants were often traveling about over those Citrus plants under examination. No attempt has been made to determine whether any of these insects are concerned in the transportation of the fungus. Available information dealing with ants and flies (1), or with ants alone (8), as carriers of other organisms pathogenic on plants, suggests, however, that these insects, and perhaps others as well as the Mycetaeid, may be concerned in the dissemination of the Citrus Sphaceloma.

It is, of course, possible that various insects are attracted to this fungus or to related fungi as food on account of the mucilaginous character of the walls and the presence of an occasional free, glistening, viscid substance[3] derived from them in which conidia may be embedded.

[3] Jenkins, A. E., (Glistening substance covering growth of *Sphaceloma fawcettii* Jenkins, *Sphaceloma ampelinum* de By., and *Plectodiscella veneta* (Sacc.) Burk.) In Fawcett, H. S., and H. A. Lee, Citrus diseases and their control. p. 490. 1926.

At any rate, observations made in connection with numerous examinations of scab lesions on young Citrus suffice to indicate that insects of one kind or another feed more or less commonly on the Sphaceloma normally present on such lesions. For instance, here and there, fully developed lesions were noticed from which some or all of the exposed growth of the fungus and even the underlying stromatic layer, as well as dead host tissue intermixed with hyphae, situated immediately beneath this layer, had prematurely disappeared as though cropped or gnawed by insects. On or about these lesions were insect feces containing free Sphaceloma conidia, as well as conidiophores and fragments of stroma presumed to be also of this



FIG. 2. Confluent lesions on leaf showing patch from which the fungus growth has evidently been eaten by insects (X 6). Photograph by J. F. Brewer.

fungus. In some cases, entire conidiophore tufts like those shown in figure 1, E, were seen, some of them still attached to the stromatic base on which they were borne. In addition, conidia and hyphae or conidiophores thought to be those of *Cladosporium* sp. were present in the feces examined, such hyphae or conidiophores often protruding beyond the rounded surfaces of these feces. The confluent lesion illustrated in figure 2 shows the appearance of Citrus-scab lesions on which insects have evidently fed. On the lower part of the patch labeled *a*, represented as small black dots, are feces of insects as present on this particular leaf of the sour orange received from Orlando late in November.

Isolations were made from two individual pellets taken from the patch illustrated in figure 2, *a*, and from each pellet the Sphaceloma was obtained in pure culture. The method of isolation was as follows: Under as nearly aseptic conditions as possible, each pellet was several times washed in an abundance of water, then macerated in a drop of water on a glass slide and examined with the compound microscope to make certain that Sphaceloma conidia were present. From this maceration, dilution plate cultures on potato-dextrose agar were made. In due time from the two sets of cultures, one from each pellet, there developed characteristic colonies of the slow growing Sphaceloma, as well as of the faster growing *Cladosporium* sp. There was little admixture of other fungi or bacteria (Fig. 1, B). The Sphaceloma isolated in this manner from feces of insects was culturally so similar to other strains of *S. fawcettii* isolated direct from lesions as to leave no question of its identity with this species.

Thus, conidial fructifications of *Sphaceloma fawcettii* on a fresh young leaf of Citrus were fed upon by a small larva classified as one of the Mycetaeids, a little-known group of beetles. Lesions of Citrus scab also were found from which some or all of the Sphaceloma growth had been eaten away by insects of one kind or another. And, from feces of insects of unknown identity present on such lesions, the Sphaceloma and *Cladosporium* sp. were isolated and grown in artificial pure culture. It must be admitted that the data here presented have not definitely proved that the particular Mycetaeid and perhaps some of the other insects here specifically named are carriers of the Citrus Sphaceloma. The assertion can be made, however, that some insect feeding on *Sphaceloma fawcettii*, the cause of Citrus scab, is evidently capable of acting as a transporting agent for this pathogenic fungus by passing it in a viable condition through the intestinal tract.

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A PROBABLE EXPLANATION OF RECENT EPIDEMICS OF BUNT IN DURUM WHEATS[1]

C. S. HOLTON[2]

Extensive studies on varietal resistance of wheats to *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn have indicated that there are wide differences in susceptibility. Gaines (1) and Stakman, Lambert, and Flor (6) called attention to the fact that, while there are great differences in the relative susceptibility of varieties within the common wheat group and some differences within the durum group, the durums as a class appeared to be more resistant than the common-wheat group in the United States.

For many years the durums seemed to be far more resistant than the common wheats in the hard red spring-wheat region of the United States, although Marquis, the most commonly grown hard red spring wheat, is highly resistant. Beginning about 1925, however, numerous complaints were made that the durums were smutting rather generally. As a matter of actual fact, bunt in durum became rather acute. It seemed to be causing greater damage in durums than in common wheats. After the outbreak of bunt in 1925, Rodenhiser and Stakman (5) suggested that it might have been due to unusually favorable soil and weather conditions for the development of bunt or to the presence of a form of the pathogene that was unusually virulent on the durums. Rodenhiser and Stakman (4, 5), Gaines (1), and Reed (2) had shown that there are physiologic forms, both of *T. tritici* and *T. levis,* which could be recognized by their virulence on certain varieties of wheat, although they found none that was especially virulent on durums. Reichert (3) called attention to the fact that in Palestine the durums actually seemed to be more susceptible to the form of bunt present there than the common wheats, which would indicate the existence of forms that attack durums severely. It seemed quite likely, in view of the increasing severity of bunt on durum varieties in the United States and in view of Reichert's observations in Palestine, that the true explanation of the outbreak of bunt in the spring-wheat region of the United States might be due to the presence of unusually virulent strains of the pathogene. Therefore experiments were made to determine whether this was true.

[1] Published with the approval of the Director of the Minnesota Agricultural Experiment Station as paper No. 898 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The writer is indebted to Dr. E. C. Stakman for suggestions and criticisms and to Dr. J. J. Christensen and Dr. H. A. Rodenhiser for suggestions offered during the course of the investigations.

It is doubtful whether the term physiologic form can be applied properly to chlamydospore collections of bunt. There seems to be evidence that many of the smuts are heterothallic; therefore, chlamydospores would represent the diploid phase, which cannot be propagated independently. When the spores germinate there presumably are reduction division and segregation of sporidia into two or more sexual groups. Hybridization would again occur before chlamydospores could be produced on the plants. If this be true, perhaps only monosporidial lines would be designated properly as physiologic forms, and the chlamydospores would be hybrids between forms. On the other hand, Tilletia may possibly be homothallic or, if heterothallic, monosporidial lines may be similar except in sex. If this be true, the objection would not hold. For practical purposes it seems justifiable to consider as physiologic forms such collections of bunt as were used in this paper; but, from the scientific viewpoint, there is some question as to whether they may properly be so designated. In any case there are decided differences in the virulence of different chlamydospore collections, and this fact has been demonstrated to have considerable significance both in the epidemiology of bunt and in the development of resistant varieties. It may be useful, therefore, to designate the different collections showing different degrees of virulence as physiologic forms, and they are so designated in this paper.

MATERIALS AND METHODS

Eight varieties of common and durum wheats and of emmer were inoculated with the spores of five collections of *Tilletia tritici*, as shown in table 1. The seed was treated with formaldehyde, washed in water, and, when dry, inoculated at the rate of 0.5 gram of spores to 100 grams of seed. After inoculation the seed was sown in triplicate, systematically distributed, eight-foot rows in the field.

The percentages of infection (Table 1) are based on counts of 900 heads in all varieties except Mindum. In this case the percentages are based on the total number of heads, ranging from 613 to 704, in three eight-foot rows. Except for Kota, Preston, and Marquis, in which smutted heads were easily detected by external examination, smutted heads were determined by clipping each head three times. All heads containing smut were recorded as smutted regardless of whether they were partially or completely so.

RESULTS

The results are summarized in table 1. The Washington, California, and Manitoba collections did not show sufficient differences in virulence on the eight varieties used to warrant separating them into distinct physiologic forms. For the present they are considered identical, although they may

TABLE 1.—*The percentage of smutted heads in eight varieties of wheat and emmer inoculated artificially with five collections of Tilletia tritici at University Farm, St. Paul, Minnesota, 1929*

Class, variety, and C. I. number	Source of inoculum and percentage of smutted heads				
	Washington	California	Manitoba	Devils Lake, N. Dakota	Litchfield, Minnesota
Common					
Kota–C. I. 5878	42.2	53.8	42.6	43.3	14.7
Preston–Minn. 924	7.4	11.6	16.1	2.3	0.9
Marquis–C. I. 3641	1.5	3.3	4.9	0.1	0.1
Marquillo–C. I. 6887	1.2	1.4	2.7	0.1	0.0
Hope–C. I. 8178	0.0	0.0	0.0	0.0	0.0
Durum					
Mindum–C. I. 5296	2.6	4.6	3.5	50.6	3.3
Pentad–C. I. 3822	12.2	8.4	8.9	41.8	9.9
Emmer					
Vernal–Minn. 1524	1.2	1.3	0.0	8.1	23.6

possibly differ in virulence on other varieties. The collection from Devils Lake, North Dakota, was extremely virulent on Mindum and Pentad (50.6 per cent and 41.8 per cent, respectively), while the remaining varieties, except Kota, were highly resistant to it. As the table indicates, the collection from Litchfield, Minnesota, shows a comparatively low degree of virulence on Kota (14.7 per cent) and a fairly high degree of virulence on Vernal (23.6 per cent). All other varieties used, except Pentad, were highly resistant to this collection. Hope was immune from all five collections.

It is obvious from the results graphically shown in figure 1 that there are three distinct physiologic forms of *T. tritici* in the five collections used. The Washington, California, and Manitoba collections were not sufficiently different in virulence to justify separating them into distinct forms and they are, therefore, considered as the same form. Rodenhiser (4) suggested that the above-named collections might be diffrent physiologic forms, but his results were not convincing enough to warrant the establishment of different forms. The fact that the Washington, California, and Manitoba collections are here considered as a distinct physiologic form does not mean that they represent a form different from form 1 or form 2, described by Rodenhiser (4), but rather that they represent a form distinct from the other two herein reported.

Undoubtedly, the collection from Devils Lake, North Dakota, is a distinct physiologic form that can be distinguished readily by its reaction on Min-

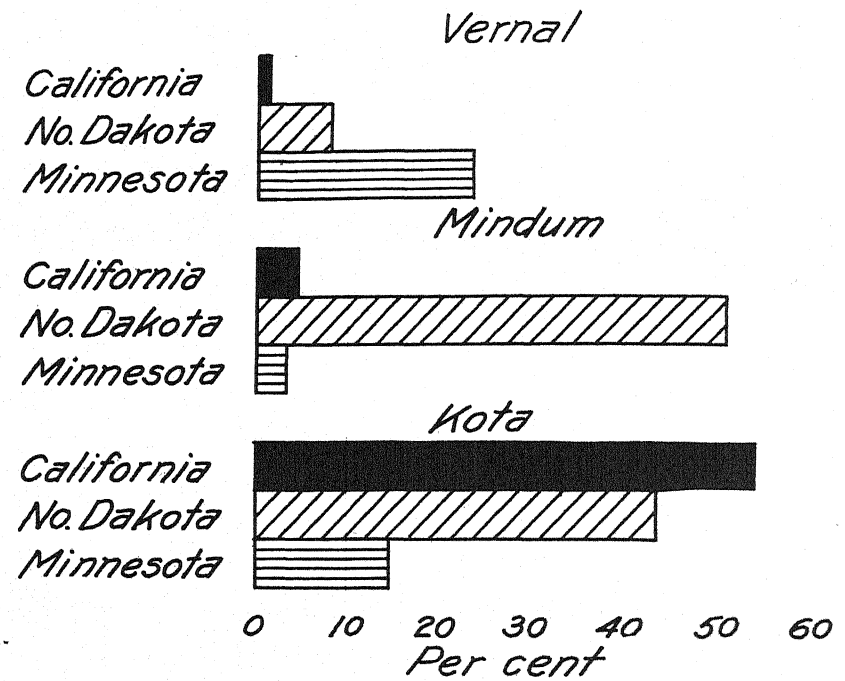


FIG. 1. The percentages of infection in Vernal, Mindum, and Kota inoculated with the spores of three collections of *Tilletia tritici* in 1929.

dum and Pentad. This form apparently was either not present or prevalent prior to about 1925. Whether it was introduced from some other region or resulted from hybridization or mutation cannot, of course, be stated.

The Litchfield, Minnesota, collection obviously represents still another physiologic form, readily distinguishable by its reaction on Kota and Vernal. Kota, extremely susceptible to the other four collections, is moderately resistant to the Litchfield form; while Vernal (emmer), resistant to all other collections of smut used in these tests, is quite susceptible to the Litchfield form. Consequently, Vernal can no longer be considered resistant to bunt.

The phenomenon of physiologic specialization in *T. tritici* is important from a practical as well as a purely scientific standpoint. One can never be certain that resistant varieties of wheat, developed for certain localities, will remain resistant indefinitely, since new physiologic forms may appear at any time and render hitherto resistant varieties completely susceptible.

SUMMARY

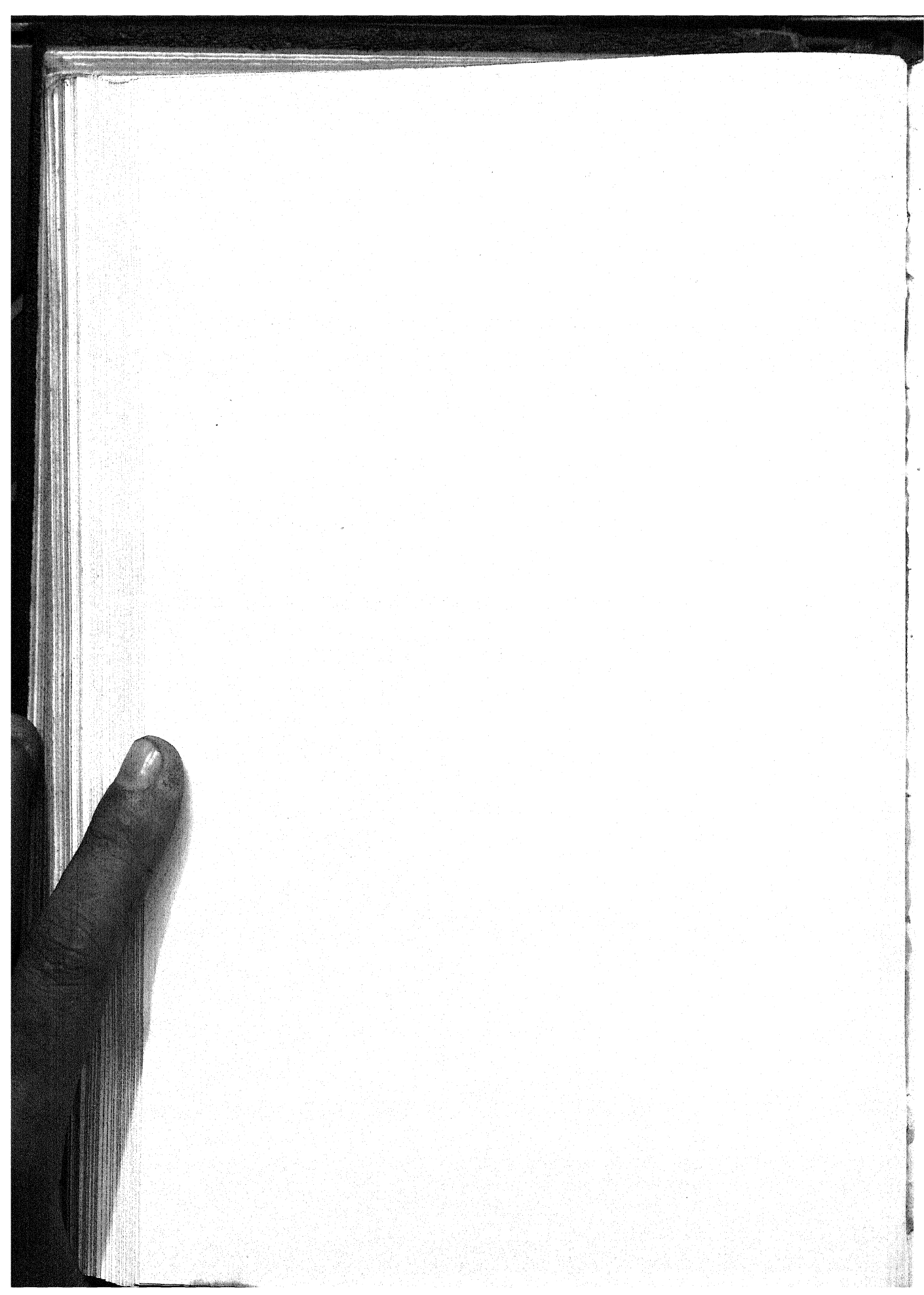
1. The virulence of five collections of *T. tritici* was compared on eight varieties of Triticum: common wheat, durum, and emmer.

2. Three distinct physiologic forms were distinguished on the basis of pathogenicity. The Washington, California, and Manitoba collections represent one form which is distinguished by its high virulence on Kota and low virulence on the other seven varieties. The collection from Devils Lake, North Dakota, represents a form characterized by its marked virulence for durum wheats, and the Litchfield, Minnesota, collection represents a third form, distinguished by its high virulence on Vernal.

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THE RELATIVE TRANSPIRATION RATE AT INFECTION SPOTS ON LEAVES

R. B. HARVEY

It has been reported by the writer[1] that the accumulation of nontoxic dyes can be used to indicate differences in the rate of transpiration from leaf surfaces. The feasibility of this method was checked by methods which, normally, should produce a high rate of transpiration in certain spots on the leaf, such as by blowing onto it a current of air from a fine nozzle and by focusing the light from a Mazda lamp on the leaf by means of a lens.

In spots over the leaf blade where, by other methods, it seems demonstrated that the transpiration rate is higher than elsewhere, there is an accumulation of Light Green S.F. and other nontoxic dyes which penetrate easily into the vascular system of the leaf. Using this dye-accumulation method it was attempted to demonstrate local differences in transpiration rate over the leaf blade and on stems in certain cases of spot infections by fungi.

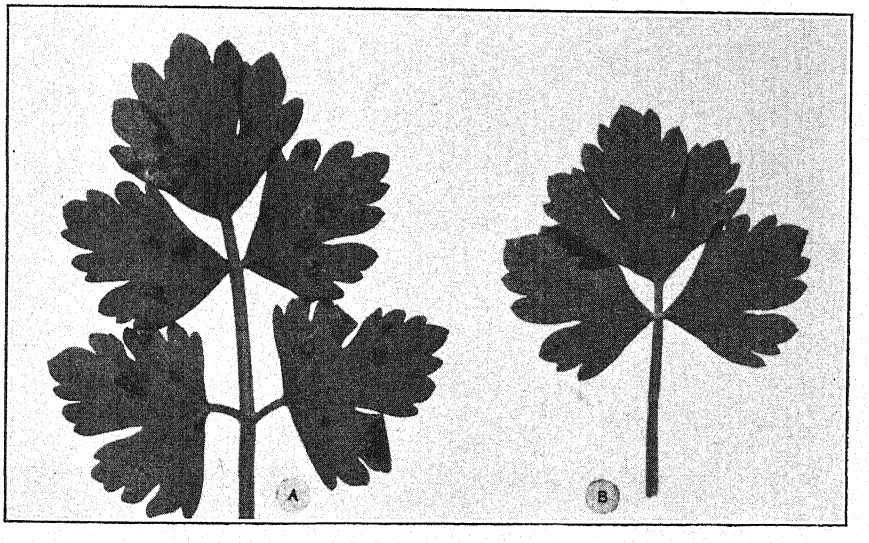


FIG. 1, A. A leaf of celery with Septoria infection spots and dye accumulation around them. Photograph through color screen to show dye in black. B. A normal celery leaf showing uniform distribution of dye. The black areas are caused by double thickness of the leaf.

[1] Proceedings of the American Society of Plant Physiologists, December, 1929.

Local infections by Septoria on celery leaves (Fig. 1, A) show dry, brown, corky areas of cells, killed by the fungus. When the petioles of infected leaves are placed in 0.1 per cent aqueous solution of Light Green S.F., or certain other nontoxic dyes, there is an accumulation around the infection spots. The brown, corky spots do not accumulate dye in a zone so much as a millimeter or so wide in the surrounding live cells. Evidently, the dead cells do not evaporate the aqueous dye solution so rapidly as do the areas into which the fungus is spreading. A leaf with no infection spots (Fig. 1, B) shows a uniform coloration of the dye.

Leaves of Hubbard squash, infected with spots of mildew (*Erysiphe* sp.) (Fig. 2), on both upper and lower surfaces, were tested in a similar

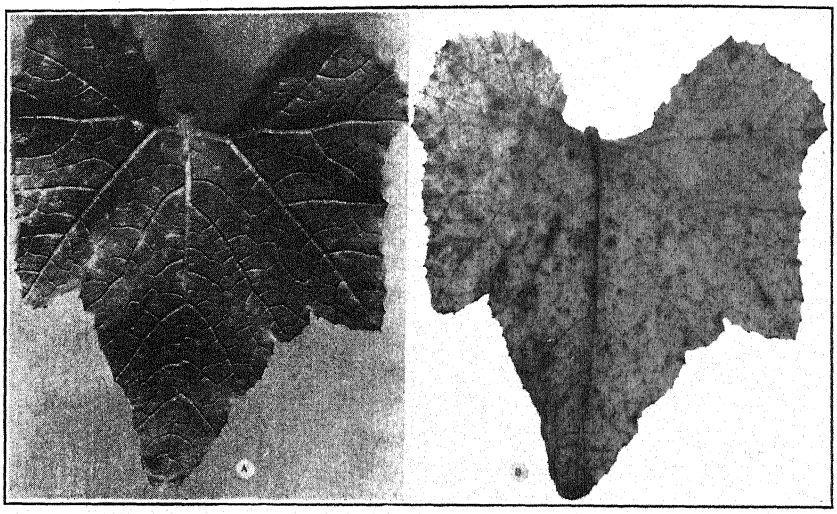


FIG. 2, A. A leaf of Hubbard squash showing areas of mildew on the lower surface. B. The same leaf photographer through a screen to show the accumulation of dye in the mildewed areas.

manner. There was no apparent correlation between the infection spots on the upper surface and the dye accumulation, but the spots of mildew on the lower surface were sharply outlined by deep-colored areas within the leaf. In this case it was evidently the presence of the fungus on the lower surface that increased the transpiration rate.

In the case of rust pustules of *Puccinia graminis* on wheat (Fig. 3, A), there is an accumulation of dye around the pustules. The color is especially deep in these areas where the epidermis is broken.

In squash leaves punctured by Aphis, the accumulation of dye was sharply limited to the affected area (Fig. 3, B), indicating that a high

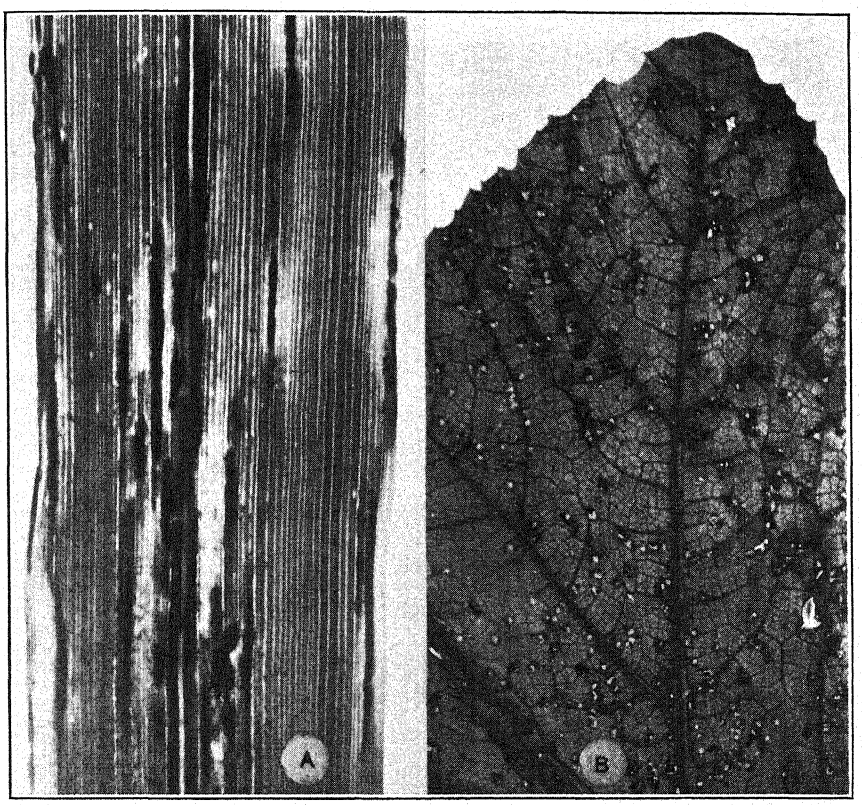


FIG. 3, A. Accumulation of dye around pustules of *Puccinia graminis* on wheat. B. A squash leaf showing insect punctures outlined by areas of dye accumulation.

transpiration rate occurred in those areas. When the leaf was ashed in the muffle furnace, there was left a perfect skeleton of the leaf structure, showing the cell walls. In the areas in which dye accumulation had occurred, there was much more abundant residue of a light grey ash than elsewhere over the leaf. This was found to be the case whether the leaves were placed in dye solution or not. Evidently the rapid transpiration at the injured spot is associated with an accumulation of ash constituents in the tissue.

SUMMARY

It is considered that the accumulation of such nontoxic dyes as Light Green S.F. can be used in demonstrating the effect of certain local infec-

tions by fungi on transpiration from the leaf. Insect punctures were found to cause local accumulations of dyes and also of ash constituents in the immediately adjacent tissue.

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PHYTOPATHOLOGICAL NOTES

The control of carnation rust, Uromyces caryophillinus (Schr.) Wint., with sulphur.—In the light of results obtained by several investigators, Kolodust, a product of the Niagara Sprayer Company, was accepted as a suitable form of sulphur. The carnation varieties used in the dusting experiments were Arctic and Radiolite. The work was carried out in a greenhouse, maintained approximately under commercial conditions. The plants were grown on standard greenhouse benches. Fertilizer was applied in the dry state and the water supplied so that the foliage never became wet or spattered. The plots were separated by double thicknesses of fine cotton cloth supported by rigid wooden frames. The dust was applied with a hand duster in quantity measured only in terms of a uniform coverage per application. The applications of sulphur were made at intervals of one, two, or three weeks. In addition, all infected leaves were hand-picked in one plot that was dusted weekly. The experimental period extended from November 27 to April 15. Thermograph records were kept from December 4 to the close of the period. In general, there were relatively few days when the temperature did not reach 70° F. for at least two hours of the 24-hour interval. In order to obtain a measure of rust infection and its spread, all plants were examined for leaf infections at the beginning and close of the experiment. The records were kept in terms of infected leaves per plot. Yield was recorded in numbers of cut flowers per plot.

The results indicate that carnation rust can be controlled by the use of Kolodust. An interval of two weeks per application is apparently as effective as one week. This accords with inoculation experiments, in which nine varieties were artificially inoculated. Rust pustules appeared in from 17 to 19 days. Data based on a limited number of controlled inoculations in nine varieties indicate a wide range in the rust resistance of carnation varieties. Dusting at three-week intervals gave no control. The weekly application, accompanied by the removal of infected leaves, proved inadequate. It is thought that hand-picking may have increased the number of infections through spore dissemination. Accepting the flower yield of the control plot as 100 per cent, the plot receiving dust at two-week intervals yielded 258 per cent. Some care was exercised to avoid dusting the petals of partially opened buds. In no case was there any noticeable direct injury to the flowers. Red spiders, *Tetranychus telarius* L. were not found on sulphur-dusted plants though they were abundant elsewhere in the greenhouse. This confirms the experience of entomologists. Near the end of the experimental period the treated plants showed signs of injury and

many individuals had died by May 15. The source of injury was found to be at the roots. Hydrogen-ion determination gave pH readings of 5.5 for the nontreated soil and 2.4, 2.9, and 3.2 for the soil receiving Kolodust at one-, two-, and three-week intervals, respectively. The rate of mortality per plot was in accord with the frequency of dust application. Since it is known that carnations are favored by applications of lime, it is believed that proper applications of agricultural lime would correct the acidity and thus protect the plants.—F. H. STEINMETZ, College of Agriculture, Orono, Maine.

The occurrence of the ring-spot disease of sugar cane in Florida.—On August 28, 1929, while the writer was making a disease survey of a small sugar-cane plot near Indiantown, Fla., typical cases of the ring-spot disease were observed on leaves of the variety D. 109. A microscopic examination of numerous perithecia, asci, and spores showed agreement in spore dimensions with published descriptions of the fungus *Leptosphaeria sacchari* van Breda de Haan. Comparison also was made with authentic herbarium material of this disease collected in April, 1923, by the writer, in Porto Rico. By these means, the identity of the disease was established beyond doubt.

Concerning the distribution of ring spot, Bell[1] has recently summarized the available information. He shows the disease present in Hawaii, Fiji, Australia, Philippines, Java, India, Mauritius, South Africa, Cuba, Porto Rico, British West Indies, Brazil, Argentina, and Peru.

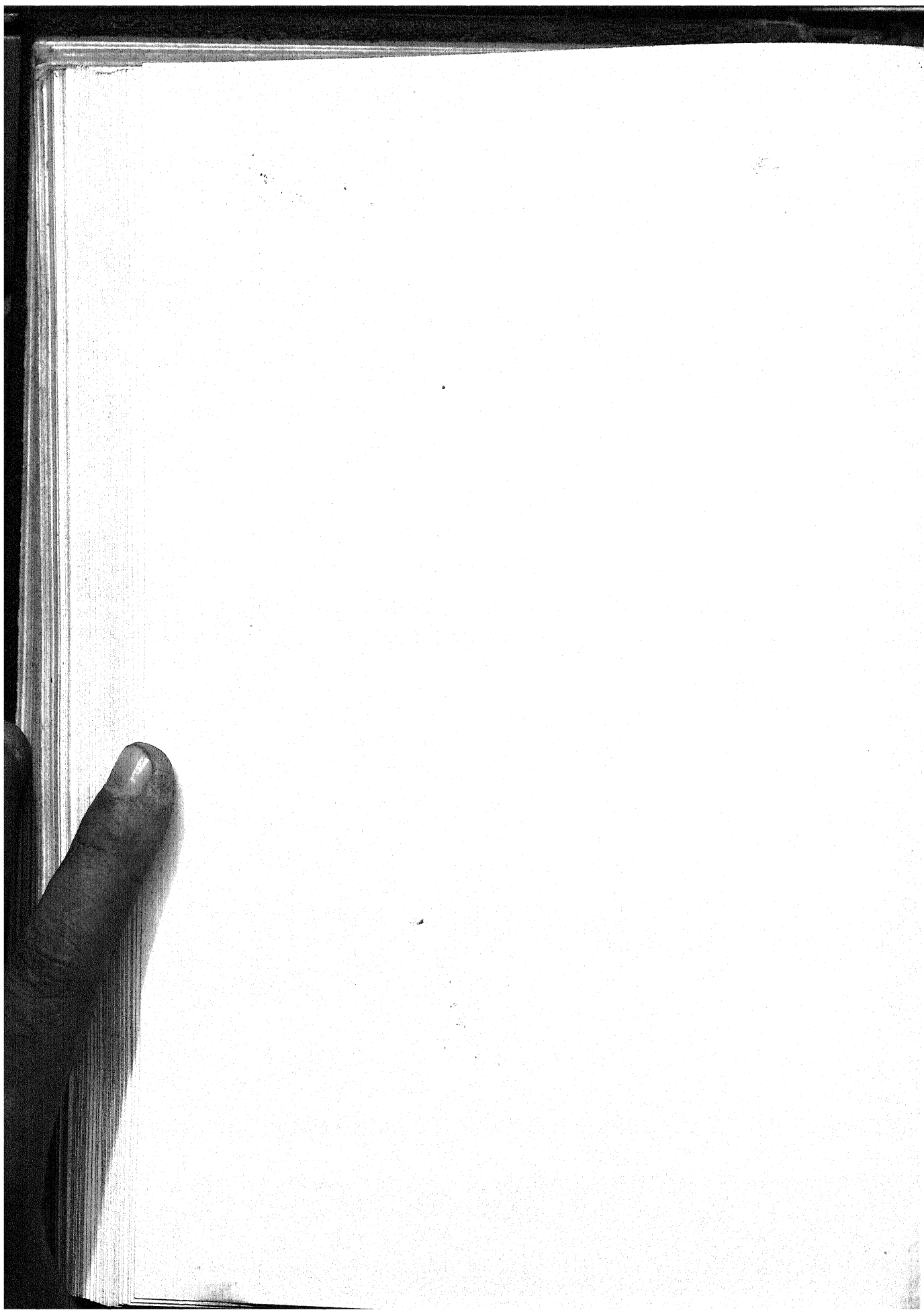
So far, the disease has been found only near Indiantown, at Canal Point, and Belle Glade, Florida. The varieties D. 109, D. 117, Co. 281, C. P. H. 139, Crystalina, and several seedlings, bred at the U. S. Experiment Station at Canal Point, have been found heavily infected, although only the lower leaves were involved and the loss at present is regarded as negligible. The disease has not been found in the large commercial sugar-cane plantings at South Bay, Clewiston, Liberty Point, and Benbow, around the south end of Lake Okeechobee. Apparently, only the regions most severely swept by the central vortex of the hurricane of September, 1928, are infested. This would seem to support the theory that living spores were carried by the hurricane from Porto Rico, where the disease is widely distributed.

Prevalence of the disease on the lower leaves of some of the noncommercial seedlings at the Florida Agricultural Experiment Station at Belle Glade makes it evident that future attention will have to be directed toward

[1] Bell, Arthur F. A key for the field identification of sugar cane diseases. Queensland Bur. Sugar Exp. Stas., Div. Path. Bul. 2. 1929.

breeding for resistance to this disease. Abbott[2] has recently recorded that two cases of severe leaf injury were reported in Peru in 1927. The plants were said to be nearly mature, however, so that only slight loss took place. Bell[1], on the other hand, has already pointed out the potential importance of the disease and cites as an example the present great economic importance of eye spot both in Hawaii and Porto Rico, due to the commercial use of certain very susceptible varieties.—B. A. BOURNE, *Pathologist, Research Department, The Southern Sugar Company, Clewiston, Florida.*

[2] Abbott, E. V. Diseases of economic plants in Peru. Phytopath. 19: 645–656. 1929.



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DEVELOPMENT OF SEED TREATMENTS FOR THE CONTROL OF BARLEY STRIPE[1]

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INTRODUCTION

The production of barley in Iowa in 1928 (27,000,000 bus.) was nearly double that of any previous year in spite of a 12 per cent loss due to barley scab and stripe. These diseases constitute serious limiting factors to successful barley production in the State. On the successful solving of this problem may depend the choice of varieties, the time of sowing, nature of fertilizer used, and the crop rotation to be followed in order to secure the most satisfactory crop returns.

The control of barley stripe (*Helminthosporium gramineum* Rabh.) may be attempted along several lines such as: (1) The selection of resistant varieties or the development of resistant strains from some commercial variety, (2) late planting in warm soils, (3) obtaining seed from regions that are known to be free from the disease, and (4) treatment of home-grown seed with some disinfectant that will control the pathogene. The first method necessitates a long-time investigation unless some variety already established is found to be resistant. The second is inadvisable for the agronomic reasons that yields are decreased and scab and rust favored by late planting. The third method involves inspection of seed in large amounts, which is a laborious and costly practice. In the fourth method, seed treatment permits the utilization of our own seed stocks, and is a desirable immediate control measure, providing we have at hand a satisfactory fungicide.

In considering seed treatment as a control measure for barley stripe, these points in the development of the disease stand out: (1) a diseased seed looks healthy, but (2) it develops into a diseased plant which rarely produces seed; (3) spores from the diseased plant go to healthy barley heads with newly forming kernels; and (4) the fungus lives over winter in the apparently normal seed from these heads.

Barley stripe is a disease particularly adapted to control by seed treatment. This is true because no plants are injured in yielding capacity ex-

[1] Published with the approval of the Director of the Iowa Agricultural Experiment Station, Ames, Iowa.

cept those grown from infected seed. Diseased plants from infected seed are almost a total loss, seldom producing seed. Therefore, if the primary and ultimately systemic infection in the seed could be inhibited or the mycelium actually killed, serious losses to the ensuing barley crop by stripe would be prevented.

Seed treatment also controls covered smut of barley but for the most part affects only indirectly such diseases as scab and rust. A good seed treatment should not injure the seed and should be inexpensive in cost of material and of labor involved in making the treatment. Of the liquid treatments, formaldehyde has shown promise, but soaking the seed is objectionable. Some progress has been made in developing dry treatments for these diseases. Rodenhiser (6) found certain dusts that were effective but was unable to obtain yield data. Leukel, Dickson, and Johnson (2, 3, and 4) recently have reviewed the literature dealing with the control of barley stripe and have mentioned the effect of dust seed treatments on yields.

This paper presents the results of studies wherein attempts have been made to devise a dust fungicide that would effectively control barley stripe and covered smut. The purpose of this paper is to show the possibility of developing good dust fungicides for the control of these diseases and to show why adequate yield data are necessary before general recommendations should be made for barley seed treatment.

LOSSES IN IOWA FROM STRIPE AND OTHER DISEASES OF BARLEY

Estimates of losses from barley stripe and other diseases in Iowa, recorded each year since 1906, are on file in the Plant Pathology Section, Iowa Agricultural Experiment Station. On the basis of averages compiled from these data, it would appear that five important diseases occur on barley in Iowa, namely, stem rust, stripe, loose smut, scab, and covered smut.

Losses reported for stem rust, *Puccinia graminis* Pers., on barley averaged 3.5 per cent, but the average loss for the past five years (1924–1928) was only 1.0 per cent. The decrease may indicate that barberry eradication as a control measure has reached a stage where it has an effect on the prevalence of this disease. There were exceptional occurrences of this disease in 1907 and 1920, when the barley crop was injured to the extent of 20.0 and 17.0 per cent, respectively.

Following stem rust, the next highest average loss, 2.7 per cent, was from stripe caused by *Helminthosporium gramineum*. This disease is not decreasing. The average loss for the past five years (1924–1928) was 3.3 per cent. Survey notes by I. E. Melhus, in 1921, state that losses caused

by stripe have been much underestimated. Data on the occurrence of stripe in our 1927 and 1929 barley-variety plots are presented in table 1. These percentages are based on actual counts.

TABLE 1.—*Occurrence of stripe plants in barley varieties grown at Mason City, Iowa, 1927, and Ames, Iowa, 1929*

Varieties	Per cent stripe plants		Varieties	Per cent stripe plants	
	1927	1929		1927	1929
Horn	0	0.25	Manchuria (Minn. 184)	1.3	2.38
Featherston	tr.	2.5	Velvet (Minn. 477)	1.3	0.94
Bonami	tr.	1.56	Alpha	2.0	3.19
Sandrel	tr.	0.56	Colsess	2.0	1.81
Trebi (C. I. 936)	tr.	0.06	Caucasian	10.0	.....
Oderbrucker (Wis. 5–1)	tr.	8.00	Manchuria (Iowa)	16.0	.....
Ontario No. 21	0.6	1.25	Minsturdi (Minn. 439)	27.0	7.69
Hero	0.6	0.31	Minsturdi	46.0	10.88
Black Hulless	0.6	0.06	Glabron	.....	1.38
Trebi (Colo.)	0.6	.....	Wisconsin Barbless	.....	0.94
			Nepal	.....	0

I. E. Melhus has collected data on the occurrence of barley stripe at Ames, Iowa, during the five-year period 1917 to 1921. With his permission a summary of these data is presented in table 2.

TABLE 2.—*Occurrence of stripe in three classes of six-row barleys grown near Ames, Iowa, 1917–1921*

Classification	Per cent stripe plants					Average
	1917	1918	1919	1920	1921	
Caucasian	10.6	6.4	2.7	9.0	11.9	8.1
Oderbrucker	5.2	3.9	1.0	4.6	10.3	5.2
Manchuria	4.7	3.4	0.3	3.9	5.7	3.6

In table 2 the percentage of stripe plants in each case is the average occurrence in several selections from that variety. The data in this table indicate that more stripe normally occurs in Caucasian than in Oderbrucker and less in Manchurian.

Loose smut, *Ustilago nuda* (Jens.) Kell. & Sw., causes an annual loss in Iowa of 1.6 per cent, but the average loss for 1924–1928 was only 0.7 per cent. The average loss each year from this disease is never high, but the consistency of its occurrence makes it an important disease of barley.

TABLE 3.—*Number of heads affected with loose smut in four rod-rows of each of eighteen varieties of barley and the approximate percentage of loose smut by varieties and by seed treatments, Ames, Iowa, 1929*

Variety	Check	Check	Ethyl Iodide	Ceresan	Bar 3	Bar 4	Pa. 10	Bow	$NaHSO_3$	Check	Check	Total number smutted heads	Approximate Pct.
Wisc. Barbless	21	22	13	5	8	8	4	6	13	10	18	128	0.7
Bonami	11	5	2	5	1	0	2	1	0	2	3	32	0.2
Oderbrucker (Wis. 5–1)	11	8	4	1	3	3	1	0	4	1	6	42	0.2
Featherston	8	9	7	5	3	3	3	6	6	5	5	60	0.3
OAC 21	1	1	0	0	0	0	0	0	2	2	1	7	0.0
Trebi	6	4	4	0	3	5	0	1	2	8	5	38	0.2
Sandrel	4	1	7	3	3	0	1	1	1	6	4	31	0.2
Horn	6	13	15	5	6	10	0	6	10	13	12	96	0.5
Velvet	8	6	6	5	4	7	7	6	9	9	6	73	0.4
Glabron	81	82	91	97	84	99	86	87	102	86	87	982	5.6
Spartan	24	14	12	14	28	14	12	14	8	14	10	164	0.9
Manchuria	4	1	1	3	2	2	2	1	2	1	1	20	0.1
Minsturdi (New)	9	5	5	2	1	5	4	6	2	2	4	45	0.3
Hero	0	1	0	3	2	1	1	2	0	2	8	20	0.1
Alpha	20	36	14	6	7	9	15	8	17	13	18	163	0.9
Colsess	162	170	106	117	108	118	114	135	121	110	136	1397	8.4
Black Hulless	13	6	6	9	6	4	4	6	10	6	9	79	0.5
Nepal	4	4	1	3	4	3	4	2	3	4	5	37	0.2
Minsturdi (Old)	5	3	7	5	5	5	5	8	3	7	3	56	0.3
Total per cent smut	1.3	1.3	1.0	0.9	0.9	1.0	0.9	1.0	1.0	1.0	1.1		1.0
Total number smutted heads	398	391	301	288	278	296	265	296	315	301	341		

The occurrence of loose smut was recorded in a barley-variety and seed-treatment experiment conducted at Ames in 1929. The percentages of smut are approximate because the rod rows were estimated to have 800 culms each. The smutted heads were counted in 836 rod rows and data are presented in table 3.

The data in table 3 show that 5.6 and 8.4 per cent of loose smut occurred in Glabron and Colsess, respectively, and less than 1 per cent in any of the other varieties. All the seed used in this experiment was obtained from one field the preceding year; therefore, these data may indicate the relative susceptibility of the varieties to loose smut. Seed treatment seems to have had some effect in the control of loose smut in the first eight varieties in table 3 and little, if any, effect on the other varieties. A summary of the smut counts in these eight varieties shows there were 58 smutted heads in the means of the checks and 11 on plants from seed treated with Pa10.

According to the Plant Disease Reporter 1927 (United States Department of Agriculture) scab of barley, caused by *Gibberella saubinetti* (Mont.) Sacc., is not considered an important disease outside of Iowa. In this State, however, there has been an estimated annual loss of 1.4 per cent. The average loss for the past five years (1924–1928) is 2.2 per cent. The occurrence is somewhat sporadic; serious damage occurred in 1919 (5.0 per cent loss) and 1928 (8.0 per cent loss). Recently scab has been charged with making barley unfit as a food for swine.

Covered smut (*Ustilago hordei* (Pers.) Kell. & Sw.) has caused an annual loss of 1.0 per cent in Iowa. The average loss for the past five years (1924–1928) was 1.3 per cent. In the United States as a whole, this disease is considered the most important of those occurring on barley. In Iowa, however, it would rank, at most, third and possibly fifth.

Barley diseases of minor importance occurring in Iowa are: Leaf rust, spot blotch, net blotch, ergot, anthracnose, powdery mildew, and bacterial blight. These probably cause a combined annual loss of about 2.0 per cent. In certain years or on certain varieties some of these diseases are important.

Spot blotch, caused by *Helminthosporium sativum* Pam. King & Bakke, is recorded (1909) as having caused 50.0 per cent loss in the two-rowed variety, Chevalier. Also there were severe infections in 1915 and 1917, injuring 10.0 per cent and 4.0 per cent, respectively.

Several times in the past 20 years net blotch, caused by *Pyrenophora teres* (Died.) Drechs., has injured the crop 5.0 per cent.

Severe infections of bacterial blight, caused by *Pseudomonas translucens* Jones, Johnson, and Reddy, have been observed in 1927 and 1928 on only one variety, Colsess. In 1929, Hero also was severely attacked.

Data are presented in table 4 on the occurrence of a number of diseases in varieties of barley grown at Ames and Mason City, Iowa, in 1929. The absence of a disease is designated by 0 in the table and the severity by

TABLE 4.—*Occurrence of a number of diseases in barley varieties grown at Ames and Mason City, Iowa, in 1929 (0 = absence; 1 = present but injury not apparent; 2 = present and caused considerable injury)*

Variety	Spot blotch	Net blotch	Scab	Stem rust	Leaf rust	Bacterial blight	Ligule blight unknown [a]	Powdery mildew
Velvet	0	1	0	2	0	0	0	0
Glabron	1	1	0	2	0	0	1	1
Wis. Barbless	0	1	1	1	0	0	1	0
Spartan	2	0	0	1	0	0	1	0
Bonami	0	1	0	1	0	0	0	0
Manchuria	0	1	1	1	0	0	0	0
Wis. 5–1	0	1	1	2	1	0	0	1
Featherston	0	1	1	1	0	0	1	0
OAC 21	0	1	1	1	0	0	1	0
Minsturdi	0	0	1	1	1	1	0	0
Trebi	1	2	2	1	1	1	2	0
Sandrel	2	2	2	1	0	1	2	0
Hero	2	1	0	1	0	2	2	0
Horn	2	1	0	1	0	0	1	0
Alpha	1	1	0	1	0	0	0	0
Colsess	1	1	1	2	0	2	1	2
Black Hulless	1	1	0	1	0	0	0	0
Nepal	1	1	2	1	0	0	0	0
Minsturdi (Old)	0	1	1	1	1	1	0	0

[a] Not scald, *Rhynchosporium secalis* (Oud.) Davis.

figures 1 to 3. 1 means that the disease was present but injury not noticed; 2, that real injury was caused; and 3, that the economic value of the crop was almost completely destroyed.

TABLE 5.—*Estimated percentage losses from barley diseases in Iowa*

Disease	Percentage loss			
	1906–1928	1924–1928	1928	1929
Stem rust	3.5	1.0	0.5	0.5
Stripe	2.7	3.3	4.0	5.0
Loose smut	1.6	0.7	tr.	1.5
Scab	1.4	2.2	8.0	tr.
Covered smut	1.0	1.3	1.0	tr.

Table 4 shows that no variety is immune from all the minor diseases. Colsess stands out as a susceptible variety, being injured by stem rust, bacterial blight, and powdery mildew. Besides these diseases, it had 1.8 per cent stripe (table 1) and 8.4 per cent loose smut (table 3) and was the only variety in which ergot was common.

A summary of the losses is presented in table 5.

METHODS AND MATERIALS

Preliminary field experiments on the control of barley stripe by seed treatments with dust fungicides were conducted at Ames, Iowa, in 1927. A large number of dusts were prepared and tested by planting the treated and untreated barley seed in rod rows. Data on the occurrence of stripe in these rows were obtained.

Fifteen of the 96 treatments tried in 1927 resulted in rod rows having no stripe plants and no plants infected with loose and covered smut. Each of the seventeen check rows had some plants affected with stripe and loose smut. The results of the experiment served to indicate chemical combinations which had disinfecting properties but apparently did not injure the seed. One combination, consisting of mercury chloride and the addition product of furfural and sodium bisulphite, gave especially promising results. Subsequent tests in the greenhouse indicated that furfural was not necessary in this combination. Fungicidal dusts of this type, with the furfural omitted, were made and used in the 1928 and 1929 field experiments.

In 1928 and 1929, the number of disinfectants was decreased and the number of field experiments was increased, so that yield data could be secured. Seed treatments were made at the rate of three ounces of dust fungicide per bushel of seed.

In one type of experiment, a barley variety and eight dust disinfectants were used. Single rod-rows were made the unit, and all treatments were replicated ten times in 1928 and 11 times in 1929. Experiments of this kind were located at Mason City, Iowa, and at two places near Ames, Iowa, *i.e.,* Agronomy Farm and the Plant Pathology plots.

In another type of experiment, 20 varieties of barley were treated with two of the dust fungicides and planted at Mason City, Iowa, in units of four rod-rows instead of one. This type of experiment was modified in 1929 by changing or omitting a few of the varieties, increasing the number of dust fungicides, and including three dates of planting at Ames and two dates of planting at Mason City.

In the first set of experiments, the number of stripe-infected plants was recorded, when the grain was in the milk stage. All the plants were pulled in one complete replication and the diseased plants separated from the

healthy ones. A rod-row thresher was used in separating the grain from the straw.

COMPOSITION OF THE DUST FUNGICIDES

Not all of the dust fungicides gave promising results. Therefore, it is necessary only to give the composition of Bar 1, Bar 3, Bar 4, P1C, and Bow.

Bar 1 was made as follows: To 2.7 grams $HgCl_2$ in solution enough NaOH solution was added to change mercury to the yellow oxide. The supernatant liquid was decanted and the yellow oxide triturated with 2 gm. $NaHSO_3$. After adding 22.3 gm. talc, it was mixed thoroughly, air dried, and pulverized.

Bar 2 was made by mixing equal parts of Bar 1 and talc.

Bar 3 was somewhat similar to Bar 1 except that the ingredients were mixed dry. It was expected that certain reactions would take place when the treated seed was sown in moist soil. Bar 3 was made as follows: 6 gm. $NaHCO_3$, 6 gm. $NaHSO_3$, 8 gm. $HgCl_2$, and 80 gm. talc were mixed and pulverized.

Bar 4 was made by mixing and pulverizing 3 gm. $HgCl_2$, 10 gm. $NaHSO_3$, and 87 gm. of talc.

P1C was made as follows: 10 gm. $HgCl_2$ and 5 c.c. cresol were placed in a mortar and triturated. Strong NaOH solution was added until the mass was yellow and thick. Strong copper-sulphate solution was added in excess of the reaction. Ninety grams of talc was added and the mixture dried and pulverized.

Bow contained 10 per cent hexamethylenetetramine, 30 per cent $CuSO_4 . H_2O$, 30 per cent $CaCO_3$, and 30 per cent talc.

EXPERIMENTAL STUDIES

It has been shown that the four diseases that probably tax the farmer most heavily are stripe, smut, scab, and rust. The first two are seed-borne diseases and are controlled at least partially and practically by seed treatment. The fact that scab and rust are most in evidence and produce the major part of their damage late in the growing season indicates that these losses may be much reduced by early maturity of the crop, thereby escaping much of the usual damage. However, early sowing, usually accompanied by cool soil temperatures, provides exceptionally favorable conditions for the seed-borne diseases, especially barley stripe.

The development of a dust fungicide to control stripe seemed to offer the most satisfactory immediate approach to the problem of barley-disease control. Field experiments were performed to determine the effect of dust fungicides on stripe and yields of barley.

TABLE 6.—*Data on the occurrence of barley stripe (H. gramineum), and yields of grain from a seed-treatment experiment using barley, variety Minsturdi, 1928*

Location of plots	Treatment	Number of plants counted		Per cent stripe plants	Yields bushels per acre	Increase following treatment	
		Healthy	Diseased			Bushels	Per cent
Mason City, Iowa	1 EE	376	19	4.9			
	2 EE2	394	69	14.9			
	3 Pb8	382	47	11.0			
	4 Bar 1	412	11	2.6	52.6	9.0	20.7
	5 Check	335	53	13.7			
	6 Bar 2	398	28	6.6			
	7 Bar 3	418	8	1.9	53.6	10.0	23.1
	8 P1C+	378	26	6.4			
	9 P1C	428	19	4.3	49.6	6.0	13.8
	10 Check	383	55	12.5	43.6		
Pathology plots, Ames, Iowa	1 EE	422	119	22.0	32.8	−1.2	−3.5
	2 EE2	444	99	18.4	32.3	−1.4	−4.2
	3 Pb8	464	46	9.0	36.8	3.4	10.2
	4 Bar 1	516	6	1.1	40.4	7.4	22.4
	5 Check	492	98	16.6	32.7		
	6 Bar 2	477	31	6.1	37.9	5.1	15.5
	7 Bar 3	503	7	1.4	41.1	8.1	24.5
	8 P1C+	492	36	6.8	36.8	3.6	10.8
	9 P1C	503	5	1.0	39.2	5.9	17.7
	10 Check	449	145	24.8	33.4		
Agronomy plots, Ames, Iowa	0 Check				29.6		
	1 EE	353	225	38.9	31.9	2.8	9.6
	2 EE2	348	190	35.3	30.7	2.1	7.3
	3 Pb8	311	151	32.7	34.4	6.2	22.0
	4 Bar 1	384	26	6.3	42.8	15.1	54.5
	5 Check	319	195	38.0	27.2		
	6 Bar 2	428	62	14.4	38.7	11.2	40.8
	7 Bar 3	418	17	3.9	41.2	13.3	47.7
	8 P1C+	405	47	10.4	37.8	9.5	33.6
	9 P1C	432	17	3.8	41.8	13.2	46.2
	10 Check	368	216	37.0	28.9		

Table 6 presents data on the occurrence of stripe plants in the variety Minsturdi and on the yields obtained from plots located at three places in Iowa in 1928. At each place there were 10 plots for each dust fungicide and 20 check plots sown with nontreated seed. One plot or replication was destroyed in obtaining the stripe counts, so that only nine replications were used in determining the acre yields.

With the use of seed from the same lot at all three places, about twice as many stripe plants occurred in the checks at the Agronomy Farm plots as in the checks at the Plant Pathology plots and about twice as many at the latter place as at the Mason City plots. The dates of planting at these three places were March 31, April 19, and April 26, respectively. Soil temperatures gradually rose from March 31 to April 26, and the results are in agreement with those of Ravn (5) and of Johnson (1) in that cool soil temperatures are favorable to the occurrence of barley stripe.

The date in table 6 also show that the Bar 1 and Bar 3 treatments, on the whole, gave the most effective control of stripe and the largest increases in acre yields. P1C treatment also increased yields, especially when the barley seed germinated under very cool soil conditions. Yield increases from these three best treatments ranged from 5.9 to 15.1 bushels, or 13.8 per cent to 54.5 per cent. The data show that stripe seriously affects yields and, that, in susceptible varieties planted early, the fungicidal control of

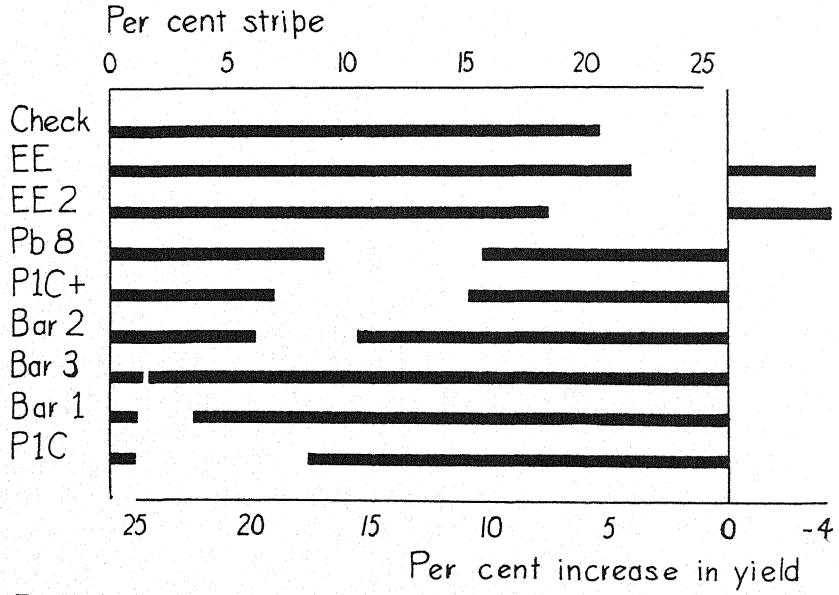


FIG. 1. Percentage stripe plants and percentage increases in yield following seed treatments of barley, Agronomy Farm, Ames, Iowa, 1928. (See Table 6.)

this disease may become one of the important practices in the successful culture of barley.

Figures 1 and 2 present graphically some of the data from the Agronomy Farm plots and the Plant Pathology plots shown in table 6. These graphs show the negative correlation between percentage of stripe plants and percentage increases in yields following seed treatments.

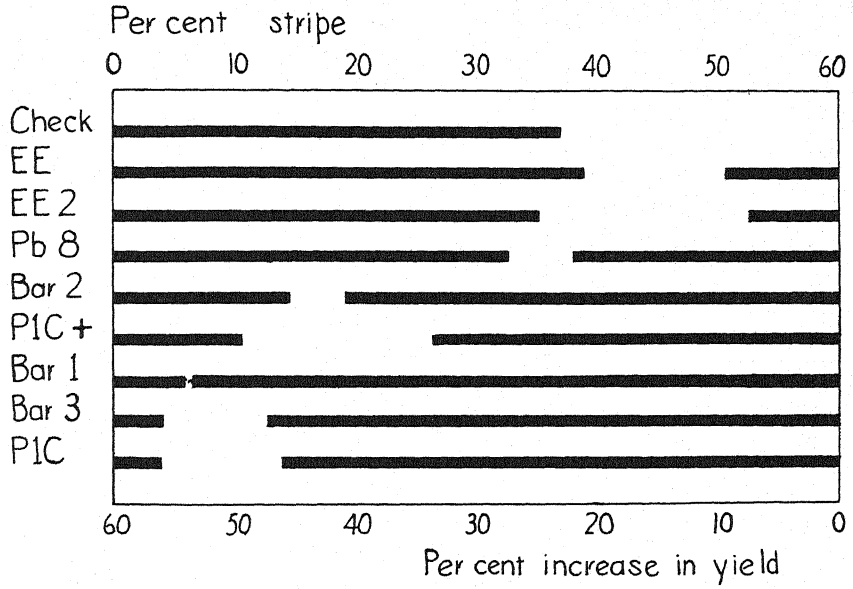


FIG. 2. Percentage stripe plants and percentage increases in yield following seed treatments of barley, Plant Pathology plots, Ames, Iowa, 1928. (See Table 6.)

Figures 1 and 2 show graphically that with each treatment used, any decrease in percentage of striped plants was followed almost invariably by a somewhat proportional increase in yield. However, when individual treatments are compared, the graphs show that Bar 1 and Bar 3 treatments were followed by larger increases than P1C treatments, although P1C treatments controlled stripe as well as or better than Bar 1 and Bar 3. It is probable that P1C was more injurious to the barley seed than Bar 1 or Bar 3.

In another experiment in 1928, using stripe-infected barley of the variety Minsturdi, 32 dust fungicides were tested in rod rows replicated five times. Yield data were obtained for 8 of these dust treatments and are shown in table 7.

In this case the results are the means of only four replications of each treatment and, therefore, are not so reliable as those shown in table 6. The

TABLE 7.—*Prevalence of barley-stripe-infected plants and the yields of grain from treated and nontreated barley seed of the variety Minsturdi grown at Ames, Iowa, 1928*

Treatments	Striped plants per rod row		Acre yields[a] in bu.		Increase	
	No.	Per cent	Treated	Checks	Bu.	Per cent
15 Check	42	8.4		36.3		
16 Corona dust 1	15	3.0	45.3	36.8	8.5	23.1
17 " " 2	8	1.6	43.0	37.3	5.7	15.3
18 16 Furfurin + 20 $CuSO_4$	14	2.8	43.2	37.8	5.4	14.3
20 Check	85	17.0		38.8		
25 Check	102	20.4		42.1		
26 Bar 4	5	1.0	49.4	41.2	8.2	19.9
28 Pb19	16	3.2	40.0	39.4	0.6	1.5
29 Hydrofuramid 28 $CuSO_4 \cdot H_2O$ 17, talc 55	14	2.8	44.4	38.3	6.1	15.9
30 Check	78	15.6		37.5		

[a] Four replications.

data are offered to show the results of the Bar 4 treatment, so that it may be compared with the Bar 1 and Bar 3 treatments in the Plant Pathology

TABLE 8.—*Prevalence of barley stripe and yields of grain from treated and nontreated seed of the variety Minsturdi, grown at Ames, Iowa, 1929*[a]

Treatment	Plants counted		Stripe plants	Increase in healthy plants following treatment	Acre yield	Increase following treatment	
	Healthy	Stripe					
	No.	No.	P. ct.	P. ct.	Bu.	Bu.	P. ct.
Barley dope	423	68	13.8	4.8	38.15	3.56	10.3
5% HgO (yellow oxide)	437	69	13.6	8.3	38.95	3.82	10.9
Sterocide	442	69	13.5	9.5	39.45	3.78	10.6
575	477	32	6.3	18.2	43.35	7.14	19.7
Check	410	136	24.9		36.75		
W 1	466	15	3.1	15.5	37.65	1.44	4.0
Bow	485	49	9.2	20.2	42.50	6.83	19.2
1234–57–2	436	66	13.1	8.1	40.80	5.67	16.1
Bar 4	468	38	7.5	16.0	42.55	7.96	23.0
Check	397	113	22.2		34.05		

[a] Date of planting, April 13.

plots, the results of which are presented in table 6. These treatments are on the same lot of seed planted side by side in the same field on the same day. Because these data indicated that Bar 4 was equal to Bar 1 and Bar 3, both in stripe control and effect on yields, Bar 4 was used extensively in experiments conducted in 1929.

TABLE 9.—*Prevalence of barley stripe and yields of grain from treated and nontreated seed of the variety Minsturdi, planted April 22 and April 30, Ames, Iowa, 1929*

Treatment	Planted April 22				Planted April 30			
	Stripe	Acre yield	Increase		Stripe	Acre yield	Increase	
	P. ct.	Bu.	Bu.	P. ct.	P. ct.	Bu.	Bu.	P. ct.
Barley dope	16.3	——	——	——	16.4	——	——	——
5% HgO	9.0	——	——	——	8.5	——	——	——
Sterocide	8.2	——	——	——	6.4	——	——	——
575	5.2	33.35	4.50	15.6	——	40.30	9.70	31.7
Check	22.4	28.85			15.6	30.60		
WB	2.1	33.40	4.55	15.8	——	37.35	6.75	22.1
Bow	8.9	——	——	——	5.9	——	——	——
1234–57–2	11.3	——	——	——	6.1	——	——	——
Bar 4	6.3	33.05	6.85	26.1	5.8	39.00	7.60	24.2
Check	21.7	26.20			17.0	31.40		

Tables 8 and 9 present data from experiments in 1929 on the variety Minsturdi, in which Bar 4 was used as a seed treatment. The three experiments here reported consisted of 11 replications of the treatments and checks, 10 of which were harvested for yields. Yield data were not obtained on some of the treatments in two of the experiments.

The data in tables 8 and 9 show that conditions for the development of stripe were about equal for the first two dates of planting, April 13 and 22, and only slightly less favorable for the third planting on April 30. The percentage of stripe was at no time so high as it was in the first planting in 1928 (Table 6). However, the soil temperatures remained comparatively low all through April, resulting in little decrease in stripe in the successive plantings. The data also show that Bar 4 did not control stripe so effectively as was indicated by the 1928 data, but the increases in yields (23.0, 26.1, and 24.2 per cent, respectively) were large in comparison with the percentage increases in healthy plants (15.2, 15.8, and 10.5 per cent, respectively).

Table 10 presents the data from two seed-treatment experiments in which the variety Minsturdi was used. The one at Ames was planted April 30 and the one at Mason City was planted May 3. Ten replications of the checks and treatments were harvested at each place.

TABLE 10.—*Prevalence of barley stripe and the yields of grain from treated and nontreated seed of the variety Minsturdi grown at Ames and Mason City, Iowa, 1929*

Treatment	Per cent stripe		Acre yields		Bu. increase		Percentage increase	
	Ames	Mason City	Ames	Mason City	Ames	Mason City	Ames	Mason City
Check	17.9	16.3	34.6	20.3				
Ceresan	0.25	0.25	39.3	23.9	5.2	3.76	15.2	18.7
1234–57–2	0.75	5.0	36.2	23.5	2.6	3.52	7.7	17.6
5% HgO	3.9	9.0	38.8	21.4	5.7	1.58	17.4	8.0
Sterocide	10.1	16.5	36.5	22.2	4.0	2.54	12.2	12.9
Check	15.3	12.8	32.0	19.5				
W 1	3.7	6.8	32.2	22.6	0.3	2.94	−0.9	15.0
Bar 4	6.2	6.0	40.1	23.5	7.0	3.68	21.2	18.6
Barley dope	15.5	12.8	37.2	23.0	3.6	3.02	10.7	15.1
Bow	3.5	9.3	34.7	21.9	0.6	1.76	2.0	8.7

The data in table 10 show that Ceresan effectively (98.5 and 98.3 per cent) controlled stripe and increased yields 15.2 and 18.7 per cent, respectively. Although Bar 4 was only 63 and 59 per cent effective in controlling stripe, the yields were increased 21.2 and 18.6 per cent, respectively.

The effect of dust fungicides on the varieties Trebi and Colsess

Table 11 presents the results of a seed-treatment experiment in 1928 on barley, variety Trebi, involving the same series of dust fungicides as we used on the variety Minsturdi. It is interesting to note that in this variety

TABLE 11.—*Acre yields from treated and nontreated barley seed, variety Trebi, grown at the Agronomy Farm in ten rod-row replications, Ames, Iowa, 1928*

Treatment	Acre yields in bushels		Increase	
	Treated	Check	Bu.	Per cent
0 Check		45.50		
1 EE	45.60	45.75	−0.15	−0.3
2 EE2	46.55	46.01	0.54	1.2
3 Pb8	48.80	46.27	2.53	5.5
4 Bar 1	48.62	46.52	2.10	4.5
5 Check		46.77		
6 Bar 2	47.50	47.06	0.44	0.9
7 Bar 3	48.35	47.36	0.99	2.1
8 P1C+	48.17	47.66	0.51	1.1
9 P1C	50.25	47.96	2.29	4.8
10 Check		48.25		

the seed did not carry stripe and, therefore, no stripe plants developed in the plot.

Table 11 shows that, although there was no stripe disease to control, the yields were not decreased by any of the treatments except EE, and this decrease was not significant. This suggests that none of the dust fungicides in the concentrations used was injurious to this variety of barley.

The yield data in table 12 were recorded from an experiment located at Mason City, Iowa, in which the same lot of barley seed was used as in table 11.

TABLE 12.—*Acre yields from treated and nontreated barley seed, variety Trebi, grown at Mason City, Iowa, in five rod-row replications, 1928*

Treatment	Acre yields	Increase	
	Bushels	Bu.	Per cent
Check	70.0		
Bar 1	69.4	−0.25	−0.36
Bar 3	78.9	9.75	14.1
P1C	63.9	−5.25	−7.6
Check	68.3		

The results presented in table 12 are not so uniform as those in table 11, probably because only five replications were harvested. However, the results are in agreement with those of other experiments in that Bar 1 and Bar 3 are more satisfactory than P1C and suggest further that P1C may be injurious to the seed.

TABLE 13.—*Prevalence of loose smut and yields of grain from treated and nontreated seed of the variety Colsess, Ames, Iowa, 1929*

Treatment	Loose smut	Acre yield[b]	Increase	
	P. ct.	Bu.	Bu.	P. ct.
1 Barley dope	5.9	41.00	2.70	7.1
2 5% HgO (yellow oxide)	6.0	38.50	0.20	0.5
3 Sterocide	5.8	43.70	5.40	14.1
4 575	5.7	43.40	5.10	13.3
5 Check[a]	5.8	38.30		
6 WB	6.2	42.00	3.70	9.7
7 Bow	5.8	39.70	1.40	3.7
8 1234–57–2	5.6	41.60	3.30	8.6
9 Bar 4	5.6	39.20	0.90	2.3
10 Check	6.4	38.30		

[a] Checks had nearly two per cent stripe.

[b] Five replications.

Table 13 presents the results of a seed-treatment experiment in 1929 on barley, variety Colsess. This variety, as shown in table 4, is susceptible to many barley diseases but not especially susceptible to stripe (1.8% in 1929).

The data in table 13 show that none of the treatments was effective in controlling loose smut but that several treatments were followed by increased yields.

RESULTS OBTAINED FROM DUST FUNGICIDES ON MANY VARIETIES OF BARLEY

Table 14 gives the results of two dust fungicides on 20 different varieties of barley grown at Mason City, Iowa, in 1928. The seed of each variety was planted in four rod-rows and there were no replications.

Table 14 shows that the mean yield of 20 varieties was slightly higher when the barley seed was treated with Bar 1 than when not treated, but the

TABLE 14.—*Yields and differences in yields due to seed treatment of barley varieties at Mason City, Iowa, 1928*

Variety	Control	Bar 1		EE	
	Yield	Yield	Difference	Yield	Difference
OAC 21	52.3	54.6	+ 2.3	53.2	+ 0.9
Minsturdi	50.2	53.8	+ 3.6	47.5	- 2.7
Trebi	62.3	75.5	+13.2	73.2	+10.9
Velvet	78.0	79.2	+ 1.2	67.5	-10.5
Comfort	70.5	75.2	+ 4.7	68.2	- 2.3
Glabron	65.5	55.2	-10.3	62.5	- 3.0
Wis. Barbless	63.7	57.5	- 6.2	58.7	- 5.0
Manchuria	56.2	51.0	- 5.2	48.5	- 7.7
Wis. No. 5–1	50.0	55.5	+ 5.5	54.5	+ 4.5
Featherston	53.5	47.7	- 5.8	61.5	+ 8.0
Alpha	59.2	56.2	- 3.0	60.5	+ 1.3
Sandrel	69.2	58.7	-10.5	70.0	+ 0.8
Hero	50.7	62.5	+11.8	54.2	+ 3.5
Horn	52.2	53.7	+ 1.5	61.7	+ 9.5
Blk. Hulless	44.5	45.0	+ 0.5	50.0	+ 5.5
Nepal	33.0	32.5	- 0.5	37.5	+ 4.5
Colsess	49.0	49.5	+ 0.5	55.0	+ 6.0
Bonami	52.5	52.2	- 0.3	50.7	- 1.8
Ia. No. 99	46.2	54.2	+ 8.0	51.5	+ 5.3
Swiss	43.5	45.5	+ 2.0	42.0	+ 1.5
Average	55.1	55.8	+ 0.7	56.4	+ 1.3
Odds			2.15:1		5.54:1

TABLE 15.—*Summary of acre yields of 18 varieties of barley in a seed-treatment and time-of-planting experiment, ten replications in all, conducted at Ames and Mason City, Iowa, in 1929*

Variety	Acre yield bushels					Increase			
	No tr.	Ceresan	Bar 3	Bow	No tr.	Ceresan	Bar 3	Bow	Mean
						Bu.	Bu.	Bu.	Bu.
Minsturdi (Old)	28.70	34.05	34.40	29.80	28.65	5.37	5.72	1.12	4.07
Minsturdi (New)	35.20	41.60	36.05	35.65	32.55	7.72	2.17	1.77	3.89
Wis. 5–1	29.70	32.95	31.10	31.75	30.10	3.05	1.20	1.85	2.03
Bonami	29.40	30.50	33.45	30.30	30.00	0.80	3.75	0.60	1.72
Glabron	34.10	36.90	37.90	33.95	35.40	2.15	3.15	−0.80	1.50
Velvet	32.80	32.60	35.50	33.15	31.85	0.23	3.17	0.82	1.41
Horn	38.85	40.25	40.75	38.75	38.20	1.72	2.22	0.22	1.39
Nepal	23.50	24.30	23.95	24.80	22.80	1.15	0.80	1.65	1.20
Black Hulless	24.55	28.30	24.75	28.45	27.40	2.32	−1.23	2.47	1.19
Colsess	34.85	34.85	36.05	35.30	33.75	0.55	1.75	1.00	1.10
Featherston	28.00	29.15	30.10	28.60	28.75	0.77	1.72	0.22	0.90
Wis. Barbless	34.75	37.30	36.00	37.65	38.75	0.55	−0.75	0.90	0.23
Manchuria	28.25	32.15	28.35	30.20	32.35	1.85	−1.95	−0.10	−0.07
Alpha	42.90	33.40	42.20	36.30	32.55	−4.33	4.47	−1.43	−0.43
OAC 21	30.60	31.45	26.55	31.45	30.20	1.05	−3.85	1.05	−0.58
Spartan	31.00	30.10	30.35	28.75	30.50	−0.65	−0.40	−2.00	−1.02
Sandrel	42.45	41.70	38.90	42.45	42.45	−0.75	−3.55	0.00	−1.43
Hero	36.15	32.00	36.40	35.65	36.85	−4.50	−0.10	−0.85	−1.82
Trebi	48.50	47.30	44.10	45.10	47.35	−0.63	−3.83	−2.83	−2.43
Av. acre yield—Bu.	33.38	34.26	34.10	33.58	33.18				
Increase—Bu.		0.98	0.82	0.30					
Increase—P. ct.		2.9	2.5	0.9					

results are not significant in terms of odds. The small increases in yields from seed treatment may be due to the fact that the seed used was almost entirely free from stripe and that only a few varieties had an appreciable amount of covered smut. The arrangement of the plot may not have been satisfactory from the standpoint of sampling the soil, because the nontreated rod rows were in one nonreplicated block. A similar block of treated rod rows was on each side of the nontreated block.

In 1929, 18 barley varieties were planted twice at early, medium, and late dates at Ames and at early and late dates at Mason City, Iowa. A variety was represented in each of the ten plantings by four rod rows from nontreated seed and seven rod rows each of which was from seed treated with a different dust fungicide. Yield data were obtained from only three of the seed treatments at Mason City. A summary of the results at both places is presented in table 15.

The data in table 15 show that, of the three treatments on which yield data were obtained, Ceresan gave the highest average increases.

Yield data were obtained at Ames on the effects of all the dust fungicides used. Here the acre yields were computed on 6 replications of each treatment and 12 replications of the checks. The plant development at Ames was more uniform than at Mason City. At the latter place, the early planting was under excessive moisture conditions and the late planting met with such severe drought as to seriously decrease the yields. It seems probable that the results of the portion of the experiment located at Ames are more significant than the results of the whole experiment. The yield data from the plots located at Ames are presented in table 16.

Table 16 shows that Trebi was the highest yielding variety and that, of the seven dust fungicides used, Bar 4 gave the highest increase when the yields of all the varieties were averaged.

Table 17 presents the acre yields of the varieties when grown at Mason City, Iowa.

The data in table 17 show that Trebi was again the highest yielding variety. A few varieties responded differently at Mason City. The variety Colsess is probably outstanding in this respect, yielding fourth from the lowest at Ames and third from the highest at Mason City.

The occurrence of stripe in plants from nontreated and treated seed of the different varieties is recorded in table 18. Stripe in the different varieties is compared with the stripe in Minsturdi, the most susceptible variety.

The data in table 18 show that Ceresan controlled stripe most effectively, followed in order by Bar 3 and Bar 4. The fungicidal dust containing 10 per cent paraformaldehyde was the most injurious to barley seed, reducing

TABLE 16.—*Mean acre yields from barley seed of 18 varieties treated and not treated and planted twice at three dates in 1929, Ames, Iowa*

Variety	Check	Ethyl iodide	Ceresan	Bar 3	Bar 4	Pa 10	Bow	$NaHSO_3$	Check	Mean acre yields of checks and treatments	Per cent yield in comparison with Trebi
Trebi	49.67	52.83	49.33	48.67	45.92	44.33	47.00	50.25	48.42	48.49	100
Sandrel	44.25	45.42	42.58	41.42	38.42	40.67	44.08	44.75	42.50	42.68	88
Horn	41.75	44.42	44.00	43.92	42.17	39.58	38.42	41.58	38.67	41.61	86
Alpha	50.67	44.92	33.67	44.00	45.25	44.42	36.58	31.67	32.67	40.43	83
Glabron	35.92	37.25	41.00	43.08	37.75	34.08	38.00	38.42	39.67	38.35	79
Hero	38.75	35.75	34.50	38.25	37.75	38.33	40.67	37.67	41.92	38.18	79
Velvet	36.50	36.42	36.00	39.67	41.83	35.67	36.08	38.67	34.42	37.25	77
Minsturdi (New)	38.67	34.17	41.83	38.58	35.83	30.50	36.58	33.67	35.25	36.12	75
Wis. Barbless	37.58	34.25	35.83	34.00	35.92	37.75	37.50	40.08	36.50	36.60	75
Wis. 5–1	35.33	34.58	40.08	34.67	38.17	32.83	36.58	34.25	36.08	35.84	74
Spartan[a]	32.88	36.13	33.00	34.25	33.13	36.88	32.00	29.88	33.38	33.50	69
Manchuria	30.83	33.50	34.67	32.50	37.92	30.17	33.17	35.33	33.67	33.53	69
OAC 21	33.75	32.92	35.67	31.50	36.58	33.83	33.00	33.67	31.75	33.63	69
Minsturdi (Old)	29.17	30.58	36.33	38.67	35.75	31.92	34.92	32.08	31.92	33.48	69
Bonami	31.00	31.25	31.42	37.17	39.92	31.83	32.17	31.92	29.17	32.87	68
Colsess	33.58	33.92	31.50	35.75	32.83	31.17	31.25	30.42	33.58	32.67	67
Featherston	30.42	31.50	34.17	30.33	34.92	30.50	32.33	33.92	31.92	32.22	66
Black Hulless	23.67	27.33	28.83	26.42	27.58	22.17	29.17	28.17	28.42	26.86	55
Nepal	23.58	25.67	24.00	24.25	23.00	20.75	25.00	21.42	23.17	23.43	48
Acre yields	35.68	35.94	36.23	36.69	36.88	34.07	35.50	35.15	34.90		
Increase—Bu.		0.34	0.73	1.29	1.58	−1.13	0.40	0.15			
Increase—P. ct.		0.95	2.06	3.64	4.48	−3.21	1.14	0.43			

[a] Only 4 replications.

TABLE 17.—*Acre yields of varieties of barley from seed treated and not treated and the mean yields from both kinds of seed compared with Trebi*

Variety	Check	Ceresan	Bar 3	Bow	Check	Mean acre yield	Per cent yield in comparison with Trebi
Trebi	53.00	44.25	37.25	42.25	45.75	44.50	100
Sandrel	39.75	40.38	35.13	40.00	42.38	39.23	89
Wis. Barbless	30.50	39.50	39.00	37.88	42.13	37.80	85
Colsess	36.75	39.88	36.50	41.38	34.00	37.70	85
Horn	34.50	34.63	36.00	39.25	37.50	36.38	82
Alpha	31.25	33.00	39.50	35.88	32.38	34.40	77
Minsturdi (New)	30.00	41.25	32.25	34.25	28.50	33.25	75
Hero	32.25	28.25	33.63	28.13	29.25	30.30	69
Glabron	31.38	30.75	30.13	27.88	29.00	29.83	67
Bonami	27.00	29.13	27.88	27.50	31.25	28.55	64
Velvet	27.25	27.50	29.25	28.75	28.00	28.15	63
Minsturdi (Old)	28.00	30.63	28.00	22.13	23.75	26.50	60
Manchuria	24.38	28.38	22.13	25.75	30.38	26.20	59
Black Hulless	25.88	27.50	22.25	27.38	25.88	25.78	58
OAC 21	25.88	25.13	19.13	29.13	27.88	25.43	57
Featherston	24.38	21.63	29.75	23.00	24.00	24.65	55
Spartan	27.25	24.25	22.50	22.25	24.75	24.20	54
Nepal	23.38	24.75	23.50	24.50	22.25	23.68	53
Wis. 5–1	21.25	22.25	25.75	24.50	21.13	22.98	52
Mean	30.21	31.21	29.98	30.62	30.53	30.50	

TABLE 18.—*Prevalence of barley stripe in 18 varieties and the effect of seed treatment on strip and acre yields (yield data from Table 16), Ames, Iowa, 1929*

Number stripe plants

Varieties	No treatment	No treatment	Ethyl iodide 5%	Ceresan	Bar 3	Bar 4	Paraform 10%	Bow	$NaHSO_3$ 15%	No treatment	No treatment	Total number	Per cent stripe in comparison with Minsturdi
Velvet	3	1	6	0	0	2	4	4	5	6	5	36	12
Glabron	8	5	6	1	3	6	13	4	6	6	3	61	20
Wis. Barbless	4	1	2	0	0	0	4	3	2	3	7	26	8
Iowa 99	5	2	3	0	2	1	6	5	5	6	5	40	13
Bonami	5	6	6	0	3	1	8	3	9	7	7	55	18
Manchuria	13	7	8	0	4	3	12	4	10	11	7	79	26
Wis. 5–1	28	36	28	0	6	16	23	22	29	31	33	252	82
Featherston	12	10	6	1	1	5	3	4	6	9	9	66	21
OAC 21	8	6	5	0	1	3	2	5	6	4	2	42	14
Minsturdi	33	33	39	1	14	19	36	31	43	25	32	306	100
Trebi	1	0	0	0	0	0	0	0	0	0	0	1	tr.
Sandrel	3	3	0	0	1	4	2	1	1	1	2	18	6
Hero	1	3	0	0	0	2	1	0	0	0	1	8	3
Horn	0	0	3	0	0	0	0	2	3	2	2	12	4
Alpha	11	13	20	0	3	8	9	7	14	11	16	112	37
Colsess	10	7	6	0	1	1	7	5	11	6	6	60	19
Black Hulless	0	1	0	0	0	0	0	0	0	0	0	1	tr.
Nepal	0	0	0	0	0	0	0	0	0	0	0	0	0
Minsturdi (old)	37	58	48	0	7	25	36	18	37	40	39	345	113
Number stripe plants	182	192	186	3	46	96	166	118	187	168	176		
Per cent stripe	2.40	2.53	2.45	0.04	0.61	1.26	2.18	1.55	2.46	2.21	2.32		
Difference in p. ct. stripe in checks and treatments			−0.08	2.33	1.76	1.11	0.19	0.82	−0.09				
Acre yield increase, p. ct.			0.95	2.06	3.65	4.48	−3.21	1.14	0.43				

the yields 3.21 per cent. It is interesting to note that, of the three treatments giving an appreciable measure of stripe control (Ceresan, Bar 3, and Bar 4), the one most effective in controlling stripe was followed by the smallest increases in yields and the one least effective was followed by the greatest increases in yields. The data also indicate that Trebi, Black Hulless, and Nepal are relatively resistant to stripe.

A summary of the effect on yields of barley varieties by seed treatment with Ceresan, Bar 3, and Bar 4, is presented in table 19. The varieties on which all three treatments increased the yields are placed in the first group in the table. The second group contains the varieties of which yields were increased by two of the three treatments, and the yields of the varieties in the third group were increased by one or none of the three treatments.

Table 19 shows that, in the first group of varieties, the highest mean increase (20.8 per cent) from seed treated with Ceresan, Bar 3, and Bar 4, respectively, was on the variety Minsturdi (old seed) and ranged down to

TABLE 19.—*Effect of seed treatment with Ceresan, Bar 3, and Bar 4 on yields of 18 varieties of barley, Ames, Iowa, 1929 (data from table 16)*

Variety	Stripe	Acre yields from seeds non-treated	Increase or decrease per acre from seed treated with			Mean increase or decrease	
			Ceresan	Bar 3	Bar 4		
	P. ct.	Bu.	Bu.	Bu.	Bu.	Bu.	P. ct.
Minsturdi (Old)	10.9	30.55	+5.78	+8.12	+5.20	+6.37	20.8
Bonami	1.6	30.09	+1.33	+7.08	+9.83	+6.08	20.2
Velvet	0.9	35.46	+0.54	+4.23	+6.37	+3.71	10.5
Horn	0.3	40.21	+3.79	+3.71	+1.96	+3.15	7.8
Manchuria	2.4	32.25	+2.42	+0.25	+5.67	+2.78	8.6
Black Hulless	0.1	26.05	+2.78	+0.37	+1.53	+1.56	6.0
Mean			+2.77	+3.96	+5.09		
Glabron	1.4	37.80	+3.20	+5.28	−0.05	+2.81	7.4
Wis. 5–1	8.0	35.71	+4.37	−1.04	+3.46	+2.26	6.3
Featherston	2.5	31.17	+3.00	−0.84	+3.75	+1.97	6.3
OAC 21	1.3	32.75	+2.92	−1.25	+3.83	+1.83	5.6
Minsturdi (New)	7.7	36.96	+4.87	+1.62	−1.13	+1.79	4.8
Nepal	0.0	23.38	+0.62	+0.87	−0.38	+0.37	1.6
Spartan	0.0	33.13	−0.13	+1.12	0.00	+0.33	1.0
Alpha	3.2	41.67	−8.00	+2.33	+3.58	−0.70	−1.7
Mean			+1.36	+1.63	+1.33		
Colsess	1.8	33.58	−2.08	+2.17	−0.75	−0.22	−0.7
Trebi	0.1	49.05	+0.28	−0.38	−3.13	−1.08	−2.2
Wis. Barbless	0.9	37.04	−1.21	−3.04	−1.12	−1.79	−5.1
Sandrel	0.6	43.38	−0.80	−1.96	−4.96	−2.57	−5.9
Hero	0.3	40.34	−5.84	−2.09	−2.59	−3.51	−8.7
Mean			−1.93	−1.06	−2.51		

6 per cent on the variety Black Hulless. The mean yields of the varieties in the second group were increased with the one exception that the mean yield of Alpha was decreased 1.7 per cent. In general, the percentage increases ranged lower than in the first group (6.3 to 1.0). The average effect of these three treatments on each of the five varieties in the third group was to decrease the yield. The percentage decreases ranged from 8.7 to 0.7. The grouping in this table tends to show that some varieties of barley benefit more than others from the same seed treatments and that some varieties may be injured by seed treatments which are beneficial to many other varieties. By using other seed-treatment materials, these same varieties might react very differently and not fall in the same groups as in table 19. In table 19, the variety Colsess is in the third group, showing that it was not benefited by Bar 4. In table 13, the data show again that there was little benefit to the variety Colsess from the Bar 4 treatment, but, in the same experiment, Sterocide materially benefited this variety.

SUMMARY

There are five important diseases of barley in Iowa, namely, (1) stem rust, (2) stripe, (3) loose smut, (4) scab, and (5) covered smut.

A satisfactory seed-treatment dust fungicide should (1) control stripe and covered smut, (2) partially control loose smut and scab, and (3) decrease the prevalence of stem rust and scab by permitting earlier planting.

In 1928, certain trial dust fungicides increased the yields from stripe-infected seed 5.9 to 15.1 bushels, or 13.8 to 54.5 per cent. Stripe was more prevalent and, therefore, yield increases were greater in the early plantings.

In 1929, in five experiments in which the plots were replicated ten times, 26.1, 24.2, 23.0, 21.2, and 18.6 per cent increase in yields was obtained by seed treatment with Bar 4 on the same lot of stripe-infected seed as was used in 1928. In two similar experiments, seed treatment with Ceresan increased the yields 15.2 and 18.7 per cent, respectively.

Data on the occurrence of stripe in 18 barley varieties from seed grown in a single field the preceding year indicate that Minsturdi and Oderbrucker (Wis. 5–1) are very susceptible and that Trebi, Spartan, Nepal, and Black Hulless are resistant varieties.

In 1929, Trebi yielded highest at both Ames and Mason City, Iowa.

Data are offered which tend to show that certain varieties may be injured by seed treatment with Ceresan, Bar 3, and Bar 4 dust fungicides.

Of the dust fungicides used in these experiments, Ceresan most effectively controlled stripe and can probably be used with safety on nearly all of the common varieties.

The results with trial dust fungicides indicate that little difficulty should be experienced in developing new ones that would result in greater increases in yields from diseased seed than are now obtained.

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THE FUNGICIDAL ACTION OF SULPHUR: I. THE ALLEGED RÔLE OF PENTATHIONIC ACID[1, 2]

FRANK WILCOXON AND S. E. A. MCCALLAN[3]

INTRODUCTION

Although sulphur and certain of its compounds have been used for many years as a means of combating fungous diseases of plants, it is remarkable that there exists today no uniformity of opinion as to how its fungicidal action is exerted; and there is scarcely a compound of sulphur which might conceivably be formed from the element, under the conditions of use, to which its toxic action has not been attributed. Among these compounds are sulphur dioxide, hydrogen sulphide, sulphuric acid, thiosulphuric acid, and pentathionic acid. Some investigators maintain that the element itself is the active agent, either in finely divided form or as vapor, although its insolubility and low vapor pressure have led the majority to consider it as a source of some more toxic principle. Extensive reviews of the earlier work on this topic are given by Windisch (54), Barker, Gimingham and Wiltshire (2), Doran (12), Young (55), Vogt (48), Thatcher and Streeter (46), Goodwin and Martin (20, 21), and others. There is a great diversity of opinion expressed, and much of the early work is inconclusive and not supported by adequate experimental evidence.

The present paper presents the results of an experimental test of the hypothesis of Young (55), first set forth in 1922, that traces of pentathionic acid associated with sulphur and formed from it constitute the active fungicidal agent. It is hoped to follow this with a second communication dealing with hydrogen sulphide, which has recently become once more of interest through the work of Marsh (30).

It has been shown by Freundlich and Scholz (19), that certain types of colloidal sulphur are stabilized by adsorbed traces of pentathionic acid. These types, called hydrophilic because of the greater degree of hydration of the particles, were found by Young (55, p. 413) to be more toxic than the hydrophobic types which do not contain pentathionic acid. Later he and Williams (51, 52, 56, 57) were able to correlate the toxicity of sulphur dusts with the presence or absence of pentathionic acid on the sulphur par-

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[3] The authors wish to acknowledge their indebtedness to Prof. C. W. Mason of Cornell University, for his optical and crystallographic observations on the samples of potassium and barium pentathionates used in this investigation.

ticles. Several of the published statements of Young in regard to the chemistry of pentathionic acid are at variance with previous chemical knowledge. For example, Young (55, p. 426) states that pentathionic acid is stable only within a narrow range of pH 4.2–5.4; whereas, it is well known that the acid is detroyed by alkalies, and rendered more stable by acids, and does not exhibit any such zone of optimum stability. Thus Freundlich and Scholz (19, p. 265), in discussing the stability of colloidal sulphur, say: "Säuren flocken deswegen so schwach, weil sie die Beständigkeit der Pentathionsäure erhöhen." Again, Young in summarizing the results of his first paper (55, p. 432) states that pentathionic acid is volatile. This is contrary to the impression of previous workers in the field of the polythionic acids, and has not been shown, so far as the present authors are aware. Young does not describe in detail his method of preparing solutions of pentathionic acid, although he and Williams (52, 57) and Williams, Liming and Young (53) give the results of spore germination tests with such solutions, and it is not clear that they ever studied pure solutions of this acid, uncontaminated by other substances. The importance of doing this will be realized when it is remembered that pentathionic acid readily reacts with many other substances forming finely divided sulphur as a product, which would complicate the interpretation of the results. The conclusions of Young have been criticized by Barker (39, p. 313; 1, p. 80) on the ground that sulphur exhibits toxicity outside the limits of pH specified by Young for the existence of pentathionic acid. Goodwin and Martin (20, p. 626) have also questioned the statements of Young regarding the volatility of pentathionic acid. Roach and Glynne (38), in their studies on the winter sporangia of *Synchytrium endobioticum* (Schilb.) Perc., were unable to find any difference in toxicity between pentathionic and sulphuric acids, at the same hydrogen-ion concentration.

In making comparisons of the toxicity of chemical substances to fungus spores, there are two requisites for obtaining accurate results which, though quite obvious, have not always received the consideration they deserve. (a) The substances whose toxicity is to be measured must be available in a pure state and of known concentration, and (b) the technique employed must be capable of distinguishing between the toxicity of the substances it is desired to compare.

EXPERIMENTAL METHODS

In these studies spore-germination tests have been employed to determine toxicity and the methods followed are those described in detail by McCallan (31, 32). These are moist-chamber tests and consist essentially of germinating the spores on glass slides in inverted moist chambers. The spores are suspended in the liquid toxic agent and pipetted as drops onto the

slides. In the case of toxic dusts, the slides are first dusted and then an aqueous suspension of spores pipetted onto the slides. The slides are supported on glass racks in the moist chamber, there being four slides to each chamber. Four drops are placed on each slide, hence there are a total of sixteen drops in each chamber. For an illustration of a moist chamber completely set up see McCallan (31). The chambers are sealed with water to preserve an atmosphere of high humidity. These chambers are placed at the desired temperature and the spores examined for germination after a given time. The percentage of germination and average length of germ tubes are recorded.

Redistilled water has been used as the medium for all tests and the temperature in all cases was within the range 19–23° C. The concentration of spores in the drops ranged from about 10 to 40 per low-power field of 1920 μ diameter. In determining the toxicity of a given compound to a given fungus, the number of spores per field was always approximately the same. The time allowed for germination before examination was from 18 to 48 hours. Since, in none of the fungus spores employed has the percentage of germination appreciably increased after 12 hours, the longer periods of time have merely facilitated greater growth of the germ tubes already formed. In most cases there was but little further elongation after 24 hours.

Four common and representative pathogenic fungi were selected for this study: *Sclerotinia americana* (Wormald) Norton and Ezekiel (13), *Botrytis* sp., of *cinerea* type, *Macrosporium sarcinaeforme* Cav., and *Uromyces caryophyllinus* (Schrank) Winter. The first three species were among those studied by Young in his first paper (55), provided that his Sclerotinia was also the common American brown-rot fungus. Young employed Sclerotinia almost exclusively in his latter studies. The *Sclerotinia americana* was isolated from infected white sweet cherries at Mattituck, New York, June, 1929. This fungus has been grown on potato-dextrose agar, where it sporulated abundantly. The spore-germination factors for *S. americana* have been discussed by McCallan (32). Since age is important, only conidia from cultures 5 to 10 days old have been used. The optimum germination temperature for these conidia is 23° C. The *Botrytis* sp., was obtained from H. H. Whetzel, Cornell University, who considers it of the *cinerea* type and designates it No. 885 in his collection. The fungus was isolated in 1927 at St. Catherines, Ontario, Canada, from marigold, and has also been grown on potato-dextrose agar, where it sporulates fairly well. The optimum germination temperature was found to be 20°–25° C. The *Macrosporium sarcinaeforme* was isolated by J. G. Horsfall at the Cornell University Experiment Station from red clover in July, 1927. This fungus was likewise grown on potato-dextrose agar and, having a wide temperature

range, the conidia germinated readily at the temperatures employed. The *Uromyces caryophyllinus* was obtained from naturally infected carnation plants of the Early Dawn variety grown in the greenhouses at this Institute. The Uromyces uredospores, as is typical of many rust fungi, do not germinate well in the centre of the drop, therefore, germination counts were confined to those in the peripheral zone of the drop. Doran's (11) narrow optimum temperature of 14° C., for the germination of these spores was not substantiated, for it was found that they germinated equally well over the range 10°, 15°, and 20° C., the latter of which was employed in these studies.

The spores from these four fungi are especially suitable because of the representative range of sulphur sensitivity each exhibits. Table 1, compiled from a number of experiments, demonstrates this varying degree of sensitivity to 300-mesh dusting sulphur.

TABLE 1.—*The degree of sensitivity to 300-mesh dusting sulphur exhibited by the spores of the fungi employed*

Fungus	Spore form	Percentage of germination		Length of germ tubes (μ)	
		Control	Sulphur	Control	Sulphur
Botrytis sp. (*cinerea type*)...	conidia	99.0	99.0	200	200
Macrosporium sarcinaeforme	conidia	99.0	99.0	400	200
Sclerotinia americana.....	conidia	98.1	60.2	400	80
Uromyces caryophyllinus.....	uredospores	84.2	11.3	400	40

Under field conditions conidia of *Sclerotinia americana* are considered very sulphur-sensitive. It is therefore probable that only those spores whose germ tubes approach the vigor and length of the control would be capable of causing infection.

Much of the published work on spore-germination toxicity tests must be discredited for two reasons: first, because of the lack of sufficiently controlled factors, and secondly, because of the lack of sufficient replications and number of spores counted. In another paper (31) the importance of controlled factors has been stressed. A thorough knowledge of the conditions affecting the germination of the fungus spores to be employed is the first prerequisite. The controls must give consistent germination if any reliance is to be placed on the results obtained. In the studies with Botrytis and Macrosporium a control germination between 97.5 and 100 per cent was always obtained. In the case of *Sclerotinia americana* seventy-five per cent

of all controls gave a germination between 96.5 and 100 per cent. When the germination percentage in the controls is unusually low a disproportionate effect is obtained with the treated spores, the percentage of germination in the latter being greatly reduced. For this reason, all experiments with *Sclerotinia americana* have been discarded in which the controls have given less than 96 per cent germination. The uredospores of the obligate parasite *Uromyces caryophyllinus* are more variable in their germination.

All experiments have been performed using duplicate and, in some cases, triplicate and quadruplicate moist-chamber tests. In general, but one microscopic field has been counted in each spore-suspension drop; however, in the case of low spore-suspension concentrations more fields have been counted. From 600 to 2000 spores have been counted from the eight slides constituting a duplicate moist-chamber experiment. The larger the number of spores counted the smaller can be the significant difference between treatments. In any experiment of this kind, it is necessary to balance the time and labor involved in counting a large number of spores against the precision attainable in the final result and effect a compromise which will give the desired precision.

From the data obtained in 250 representative duplicate tests, the standard deviation of a single moist-chamber test was calculated by the method of Fleisch (14), and that of the difference between two such tests by the usual formula $\sigma^2_{A-B}=\sigma^2_A+\sigma^2$. The significance of any observed difference between two treatments may then be estimated by referring to a table of values of the probability integral. Unless the difference is at least twice its standard deviation it can not be considered very significant. These values, calculated for five different germination-percentage classes, are shown in table 2.

TABLE 2.—*The average percentage of deviation of representative duplicate tests from their mean, and the difference in germination percentage required to show a significant difference between treatments, calculated for five germination-percentage classes*

Germination—percentage class	Total number duplicate tests	Average percentage of deviation of duplicates from their mean	Standard deviation of single test: per cent	Standard deviation of difference between two similar tests: per cent	Germination—percentage difference to give odds of 50–1
0– 20	50	0.99	2.27	3.21	7.45
20– 40	50	2.60	4.80	6.79	15.75
40– 60	50	3.78	7.46	10.55	24.48
60– 80	50	2.76	5.61	7.93	18.50
80–100	50	0.45	1.19	1.68	3.90

It will be observed that the mean deviation of duplicates is least in the 0–20 and 80–100 per cent groups, and greatest in the 40–60 per cent group. Because of the variation in the viability of the spores, a repetition of tests at different times, that is different experiments, will not give such consistent results as duplicates of the same experiment at the same time. A similar effect has been observed by Smith (42, p. 31).

THE TOXICITY OF PENTATHIONIC ACID

The chemistry of pentathionic acid

Historical.—Pentathionic acid, $H_2S_5O_6$, has been the subject of numerous investigations (17, 18, 28, 29, 41) since its discovery by Wackenroder (49) more than eighty years ago, in the solution obtained by passing hydrogen sulphide and sulphur dioxide into water. The difficulties encountered in preparing the salts of this acid in pure form suitable for analysis led some workers to doubt its existence. Spring (44, 45) in a series of papers, the last of which was published in 1882, presented a critical discussion of previous work and concluded that the alleged pentathionic acid was a solution of sulphur in tetrathionic acid. In 1888 Debus (10) published the results of a very thorough study of the Wackenroder solution in the course of which methods were devised for obtaining several of the salts in pure form, by treatment of the concentrated solution with the acetate of the metal whose salt was desired. It was also shown that the salts could be recrystallized from an acidulated solution with less decomposition than from pure water. The work of Debus eliminated all doubt as to the existence of the acid, and subsequent investigators have dealt with the mode of its formation and decomposition (16, 24, 35, 36), structure, physical properties (15, 23), and its quantitative determination in the presence of other sulphur acids (25, 27, 37). Some of these questions have not yet been satisfactorily settled (3). It was found by Salzer (40) that pentathionic acid was formed in an acidified solution of sodium thiosulphate if a small amount of arsenious acid were present. This method of preparation of the acid and its salts has been used by Raschig (34) and has been thoroughly studied by Kurtenacker and Czernotzky (26). It furnishes a much more convenient method of preparation than the original method of Wackenroder, since, by suitable adjustment of the concentrations of the reactants, the yield of pentathionic acid can be made large and that of other polythionic acids quite small.

In addition to these two methods of preparation it has been shown that pentathionic acid is formed by the hydrolysis of sulphur monochloride (19, p. 266), as well as by leading sulphur vapor and water vapor through a heated tube (22). Of special interest in connection with this investigation

is the suggestion of Brugnatelli and Pelloggio (6) that oxidation of sulphur in the presence of moisture leads to the formation of pentathionic acid as an intermediate product in the formation of sulphuric acid. An interesting case of the occurrence of pentathionates in nature is reported by Day and Allen (9, pp. 115–118). These authors found that salt incrustations around hot springs near Lassen Peak, Shasta County, California, contained considerable amounts of soluble pentathionates.

Pentathionic acid has not been obtained in a pure state, although aqueous solutions containing 50–60 per cent of the acid may be prepared. It is a strong acid comparable to sulphuric acid and may be titrated with methyl-orange indicator. It is fairly stable in the presence of other strong mineral acids, but is at once decomposed by addition of an excess of sodium hydroxide solution, with separation of elementary sulphur in finely divided form, and this reaction forms the basis of a characteristic qualitative test for pentathionic acid, since no other oxygen acid of sulphur gives this test, with the possible exception of the recently prepared hexathionic acid (50). A less conclusive test is the formation of silver sulphide when a solution containing pentathionic acid is treated with ammoniacal silver-nitrate solution.

Among other reactions of pentathionates may be mentioned that with sodium sulphite forming sodium trithionate and thiosulphate, which has been used as a rapid titration method by Kurtenacker and Bittner (25). Hydrogen sulphide also rapidly decomposes pentathionic acid forming sulphur and water, according to Debus (10).

Preparation of potassium pentathionate and barium pentathionate.—In order to test the hypothesis that pentathionic acid is a factor in the toxic action of sulphur on fungus spores, it is desirable to prepare solutions of the acid from its purified salts, since the crude preparations obtained by the method of Wackenroder or of Salzer may contain a number of other compounds in addition to pentathionic acid. The potassium salt has been prepared by a number of investigators and its optical and crystallographic properties described by Fock and Klüss (15). Barium pentathionate has been prepared by Lenoir (29) and by Fordos and Gélis (18), and these authors note that the barium salt when precipitated by alcohol persistently retains small amounts of alcohol.

The procedure used in this investigation was the same as that described by Raschig (34, pp. 274–289). The final syrupy solution of pentathionic acid was freed from sodium pentathionate as described by him and treated with potassium acetate and acetic acid in alcohol. The potassium salt was recrystallized once from water containing 1 per cent sulphuric acid, washed with alcohol, and dried over calcium chloride. In the case of the barium salt, a concentrated solution of barium acetate, containing 3 per cent of free acetic acid was added to the pentathionic acid and barium sulphate filtered

off. On treating the filtrate with an equal volume of alcohol, the barium pentathionate separated in well-formed rectangular tablets. This salt was dissolved in water acidulated with acetic acid, and reprecipitated with alcohol. The proportion of potassium or of barium in these preparations was determined by ignition to constant weight with an excess of concentrated sulphuric acid and that of sulphur by oxidation with bromine and hydrochloric acid followed by precipitation as barium sulphate.

TABLE 3.—*Results of analyses of potassium pentathionate and barium pentathionate*

Potassium pentathionate			Barium pentathionate		
	Found	Calculated for $K_2S_5O_6.1\frac{1}{2}H_2O$		Found	Calculated for $BaS_5O_6.3\frac{1}{2}H_2O$
Potassium ...	21.67%	21.63%	Barium	30.21%	30.08%
Sulphur	44.16%	44.34%	Sulphur	35.30%	35.10%
Ratio K–S...	2–4.97	2–5	Ratio Ba–S...	1–5.01	1–5

The crystals dissolved in water, formed a clear solution and gave the qualtative tests for a pentathionate, *i.e.*, an immediate precipitation of sulphur with sodium hydroxide and a brownish black precipitate with ammoniacal silver nitrate. A 0.1-gram portion in 10 c.c. did not decolorize 1 drop N/10 iodine, indicating the absence of sulphites and thiosulphates, and gave no precipitate with barium chloride, indicating the absence of sulphates. The crystals (Figs. 1 and 2) were submitted for examination to C. W. Mason, of Cornell University, who reports as follows:

"Potassium pentathionate hemitrihydrate. Orthorhombic, (as described by Groth: Chemische Krystallographie **2**: 717. Leipzig, 1906). 2V about 65°, $v < \rho$, with the axial plane parallel to 010. Optically negative. Refractive indices by the immersion method: $\alpha = 1.570$; $\beta = 1.63 \pm$; $\gamma = 1.658$."

"Barium pentathionate, hydrated. As received: well-formed rectangular tablets, occasionally with their corners truncated by small bipyramid faces. The tablets are flattened parallel to the axial plane, so that only edge views exhibit interference figures. 2E is large, and the optical character is positive (+). Refractive indices by the immersion method: $\alpha = 1.620-$; $\beta = 1.640-$; $\gamma = 1.670$."

"The above tablets, placed in water, become covered by numerous small prismatic crystals. Recrystallized from water, in which the salt is very soluble, imperfect tapering four sided prisms with curved edges, very acute ends, and marked transverse cleavage, are obtained. These exhibit extinction varying from parallel to about 8° as a maximum. Interference figures

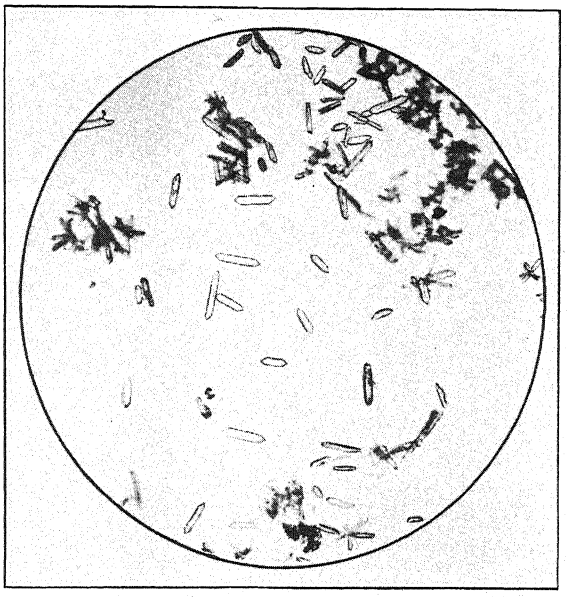


FIG. 1. Crystals of potassium pentathionate from aqueous solution by addition of alcohol.

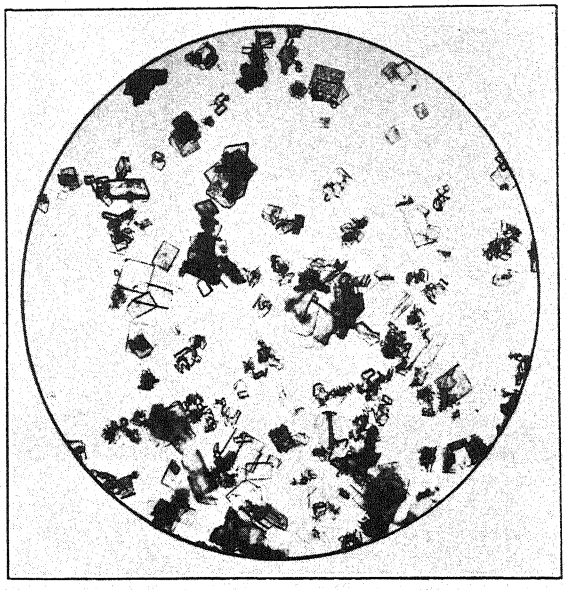


FIG. 2. Crystals of barium pentathionate from aqueous solution by addition of alcohol.

were not obtained on account of the unfavorable habit of the crystals. Approximate refractive indices, by the immersion method: $\alpha = 1.59$; $\beta = 1.67$; $\gamma = 1.75$. Recrystallized by the addition of alcohol to a concentrated aqueous solution, crystals like those from water are first formed, singly and in clusters. As more alcohol is added these dissolve and rectangular tablets appear, finally replacing them completely. This indicates the existence of two different degrees of hydration."

Solutions containing pentathionic acid were obtained by treating 1 gram of the potassium salt with the calculated quantity of tartaric acid required to liberate the pentathionic acid and form potassium acid tartrate, if the reaction were complete. It was found, however, by weighing the potassium acid tartrate which crystallized out, that the conversion was approximately 50 per cent complete and the final solution contained pentathionic acid, potassium pentathionate, and tartaric acid.

In the case of the barium salt, solutions of pentathionic acid were obtained by adding to a known solution of the salt the exact quantity of standard sulphuric acid required to precipitate the barium as sulphate. The latter was filtered off and the solution diluted to the concentration desired. The pentathionic acid solutions obtained in this manner were quite stable, did not deposit sulphur on standing, and still gave tests for pentathionic acid after keeping for a month.

The relative toxicity of pentathionic acid, sulphuric acid, and hydrogen sulphide

In this series of experiments, the comparative toxicity of pentathionic acid, sulphuric acid, and hydrogen sulphide was determined. Sulphuric acid was chosen for comparison because it is a typical strong mineral acid, as is pentathionic acid, but the sulphate ion shows no marked toxicity to fungus spores (7). Sulphuric acid is commonly present in commercial sulphur, and in greater quantities than pentathionic acid, and finally, it was used as a standard of comparison by Young and his coworkers (52, 53). Preliminary results with hydrogen sulphide are included, since the recent work of Marsh (30) appears to confirm the early findings of Polacci (33) that this substance is an important factor in the fungicidal action of sulphur. Hydrogen sulphide rapidly escapes from aqueous solutions exposed to the air. Hence it was necessary to perform the toxicity tests in a closed vessel. The hydrogen-ion concentration of the sulphuric acid and pentathionic acid solutions was determined with the antimony electrode (4, pp. 83, 84), because irregular results were obtained with pentathionic acid when the hydrogen electrode was used. The results of this series of experiments are presented in tables 4 to 8 and figures 3 to 10.

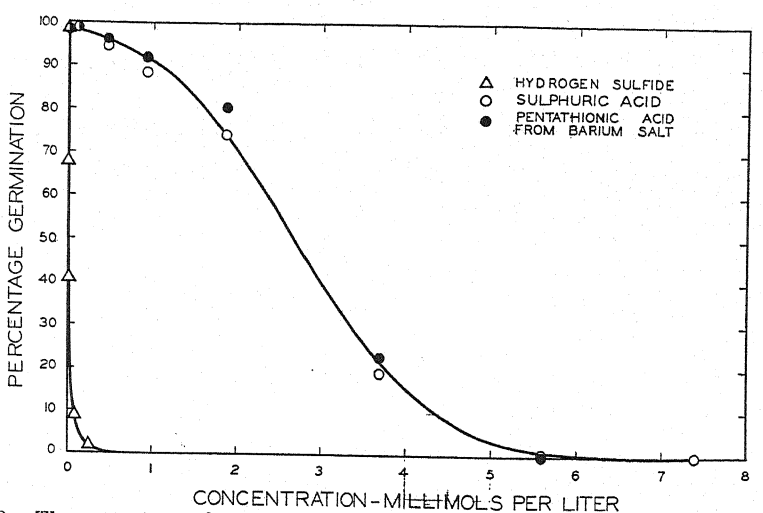


FIG. 3. The percentage of germination of conidia of *Sclerotinia americana* in solutions containing varying concentrations of H_2S, H_2SO_4 and of $H_2S_5O_6$ prepared from the barium salt.

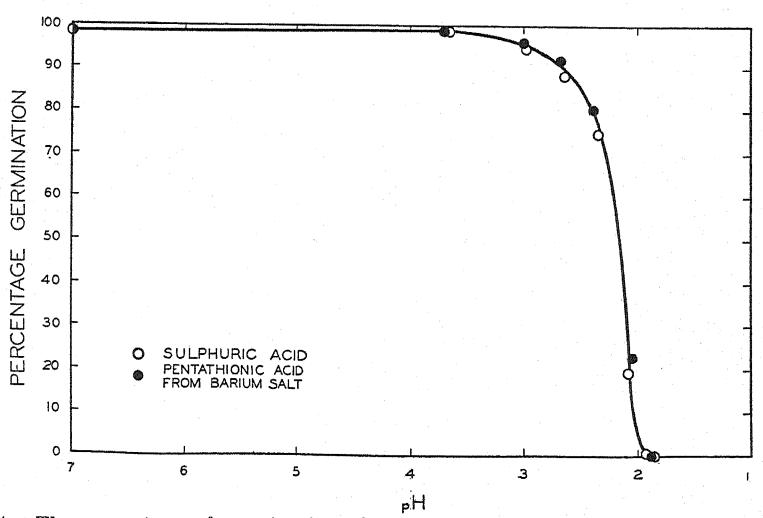


FIG. 4. The percentage of germination of conidia of *Sclerotinia americana* in solutions of H_2SO_4 and $H_2S_5O_6$, plotted against the pH of these solutions.

TABLE 4.—*The comparative toxicity of pentathionic acid and sulphuric acid solutions to the conidia of Sclerotinia americana*

Pentathionic acid (from barium salt)				Sulphuric acid			
Concentration (millimols per litre)	pH	Percentage germination	Germ-tube length (μ)	Concentration (millimols per litre)	pH	Percentage germination	Germ-tube length (μ)
Control	——	98.4	300	Control	——	98.4	300
0.092	3.70	98.6	300	0.092	3.65	98.7	320
0.462	3.00	96.0	250	0.462	2.98	94.7	225
0.930	2.67	91.9	175	0.930	2.64	88.2	150
1.860	2.38	80.3	100	1.860	2.35	74.9	80
3.696	2.04	22.9	40	3.696	2.07	19.3	35
5.604	1.88	0	0	5.604	1.92	0.5	25
——	——	——	——	7.392	1.86	0	0

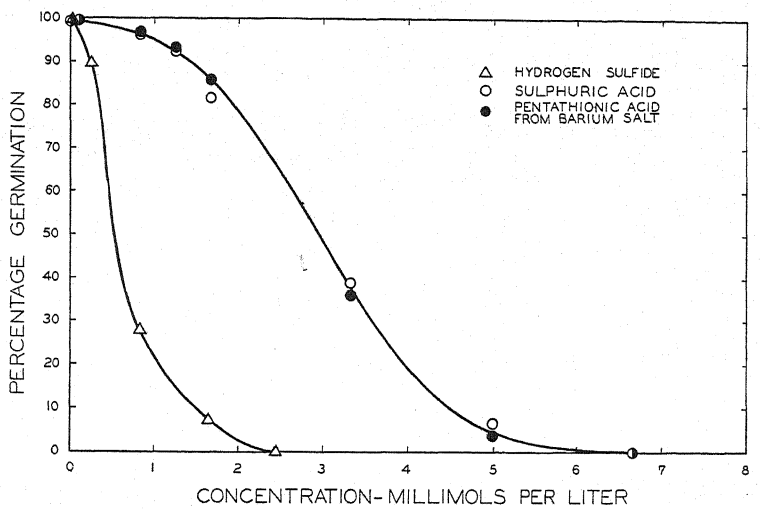


FIG. 5. The percentage of germination of conidia of *Botrytis* sp. (*cinerea* type) in solutions containing varying concentrations of H_2S, H_2SO_4 and of $H_2S_5O_6$ prepared from the barium salt.

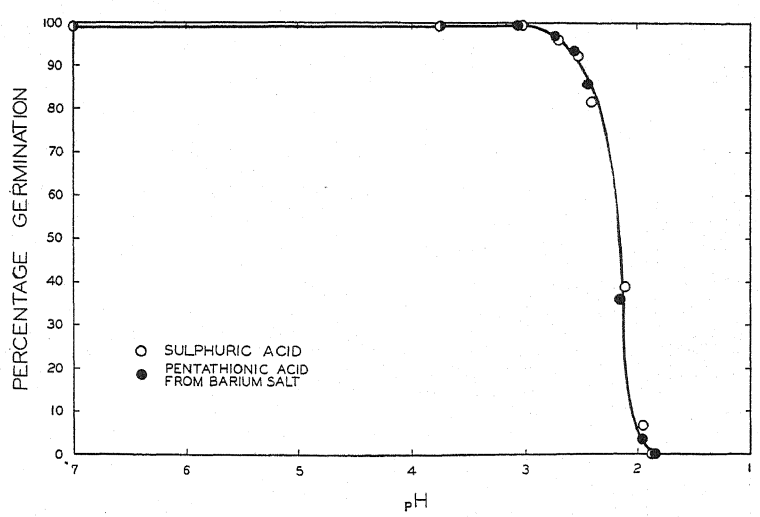


FIG. 6. The percentage of germination of conidia of *Botrytis* sp. (*cinerea* type) in solutions of H_2SO_4 and $H_2S_5O_6$, plotted against the pH of these solutions.

TABLE 5.—*The comparative toxicity of pentathionic acid and sulphuric acid solutions to the conidia of Botrytis sp. (cinerea type)*

Pentathionic acid (from barium salt)				Sulphuric acid			
Concentration (millimols per litre)	pH	Percentage of germination	Germ-tube length (μ)	Concentration (millimols per litre)	pH	Percentage of germination	Germ-tube length (μ)
Control	—	99.0	200	Control	—	99.0	200
0.084	3.75	99.3	200	0.084	3.75	99.3	200
0.418	3.06	99.3	200	0.418	3.03	99.3	200
0.835	2.72	96.8	150	0.835	2.70	96.0	150
1.254	2.54	93.2	100	1.254	2.52	92.2	100
1.669	2.43	85.7	85	1.669	2.40	81.5	75
3.332	2.15	35.9	45	3.332	2.11	38.9	50
4.998	1.95	3.4	25	4.998	1.95	6.3	30
6.664	1.85	0	0	6.664	1.87	0	0

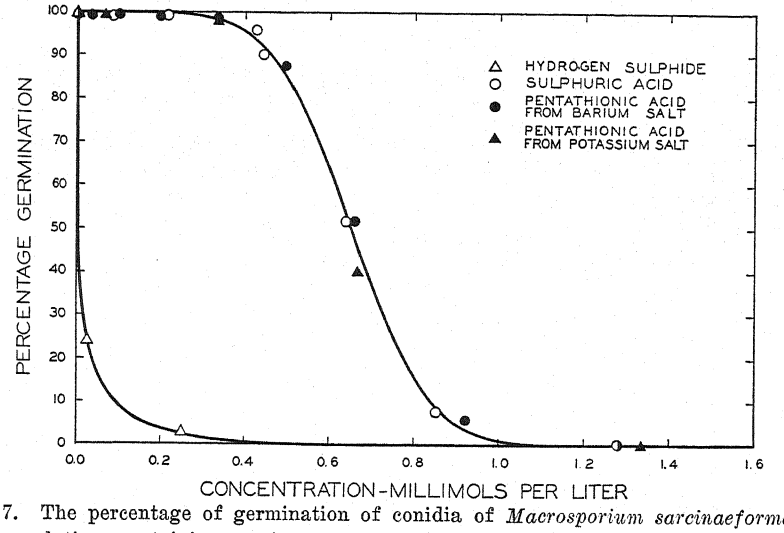


FIG. 7. The percentage of germination of conidia of *Macrosporium sarcinaeforme* in solutions containing varying concentrations of H_2S, H_2SO_4 and of $H_2S_5O_6$ prepared from the barium and potassium salts.

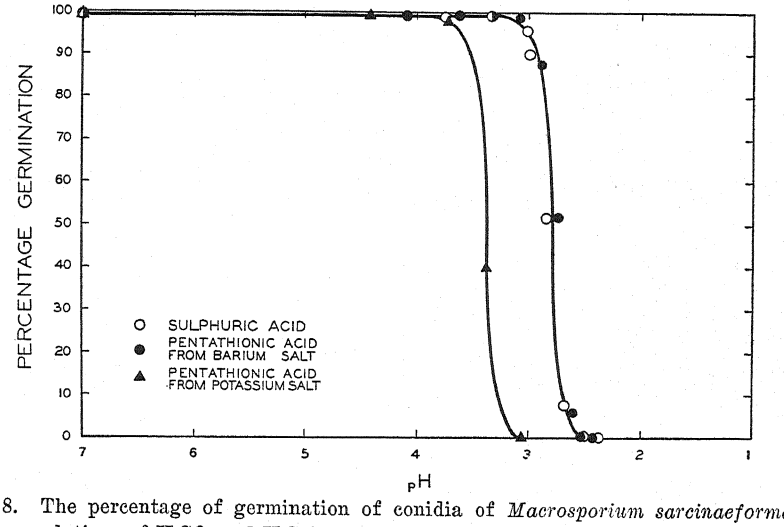


FIG. 8. The percentage of germination of conidia of *Macrosporium sarcinaeforme* in solutions of H_2SO_4 and $H_2S_5O_6$ plotted against the pH of these solutions.

TABLE 6.—*The comparative toxicity of pentathionic acid and sulphuric acid solutions to the conidia of Macrosporium sarcinaeforme*

Pentathionic acid								Sulphuric acid			
From barium salt				From potassium salt							
Concentration (millimols per liter)	pH	Percentage of germination	Germ-tube length (μ)	Concentration (millimols per liter)	pH	Percentage of germination	Germ-tube length (μ)	Concentration (millimols per liter)	pH	Percentage of germination	Germ-tube length (μ)
Control	—	99.3	400	Control	—	99.0	400	Control	—	99.3	400
0.033	4.08	99.1	500	0.006	5.50	99.0	400	0.084	3.75	98.8	425
0.099	3.62	99.2	500	0.064	4.42	99.0	400	0.214	3.34	99.0	400
0.199	3.33	99.0	500	0.333	3.73	97.7	250	0.426	3.02	95.7	300
0.332	3.07	98.7	500	0.667	3.40	40.0	120	0.437	3.00	90.0	300
0.497	2.88	87.7	300	1.333	3.08	0	0	0.640	2.83	51.6	125
0.663	2.73	51.8	200	——	——	——	——	0.853	2.68	7.5	30
0.924	2.60	5.9	30	——	——	——	——	1.280	2.51	0.2	15
1.280	2.53	0.2	15	——	——	——	——	1.706	2.38	0	0
1.710	2.42	0	0	——	——	——	——	——	——	——	——

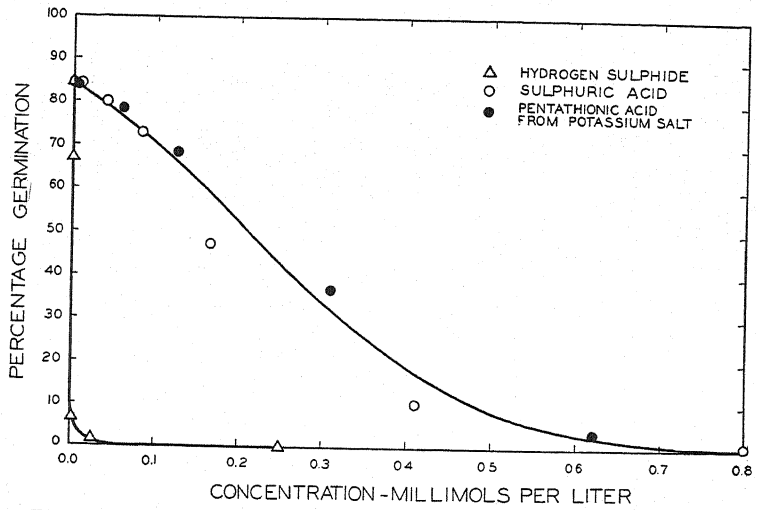


FIG. 9. The percentage of germination of uredospores of *Uromyces caryophyllinus* in solutions containing varying concentrations of H_2S, H_2SO_4 and of $H_2S_5O_6$ prepared from the potassium salt.

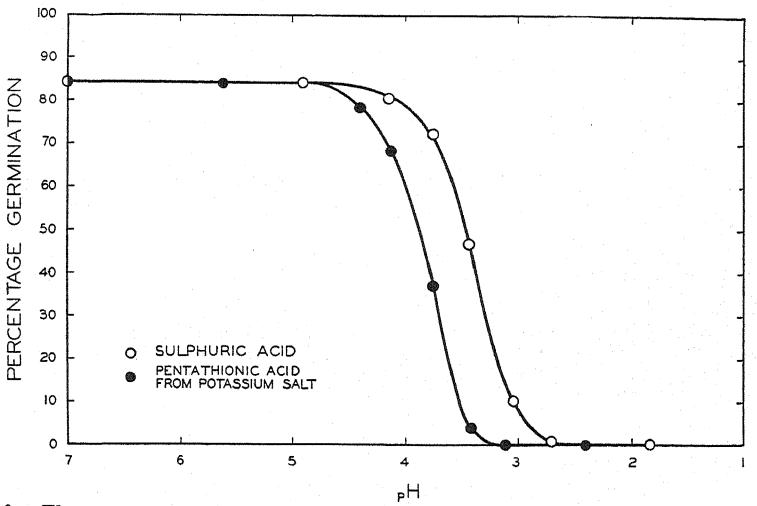


FIG. 10. The percentage of germination of uredospores of *Uromcyes caryophyllinus* in solution of H_2SO_4 and $H_2S_5O_6$, plotted against the pH of these solutions.

TABLE 7.—*The comparative toxicity of pentathionic acid and sulphuric acid solution of the uredospores of Uromyces caryophyllinus*

Pentathionc acid (from potassium salt)				Sulphuric acid			
Concentration (millimols per litre)	pH	Percentage of germination	Germ-tube length (μ)	Concentration (millimols per litre)	pH	Percentage of germination	Germ-tube length (μ)
Control	—	84.2	400	Control	—	84.2	400
0.006	5.60	83.8	400	0.008	4.90	84.2	400
0.062	4.40	78.1	300	0.041	4.15	80.0	300
0.124	4.12	68.5	200	0.082	3.75	72.7	200
0.311	3.76	36.9	150	0.163	3.45	46.9	150
0.622	3.43	3.9	50	0.408	3.05	10.1	75
1.245	3.12	0	0	0.816	2.71	0.8	25
6.224	2.42	0	0	8.157	1.84	0	0

Since the results obtained with a given fungus, using sulphuric and pentathionic acids, are in no case significantly different, as was also found by Roach and Glynne (38), working with *Synchytrium endobioticum,* a single curve has been drawn through all the points obtained. This curve was derived by plotting the points on probability paper (47, pp. 263–270) and drawing the best straight line through the points by the method of least squares. The sigmoid curve obtained in this way, which may be considered as the integrated form of a probability curve, appears to fit the data quite well. The theoretical significance of such a curve in relation to toxicity experiments has been discussed by Brooks (5, p. 78) and Smith (42, pp. 31–34; 43, pp. 340–341). It will be observed that in every case hydrogen sulphide exhibits much greater toxicity than either sulphuric acid or pentathionic acid.

When the percentage of germination is plotted against pH for sulphuric and pentathionic acids, no evidence is obtained for an optimum zone of toxicity. The solutions show no effect until a rather high acidity is reached, which varies for the different species of fungi, and the curves then drop sharply, the point of complete inhibition of germination being reached quite rapidly.

The difference in effect of the pentathionic acid solutions made from the potassium salt and those made from the barium salt may perhaps be ascribed to the content of foreign material in the former, namely, potassium pentathionate and tartaric acid, which contribute much to the total molecular concentration but little to the acidity.

An interesting observation frequently made was that discarded solutions of pentathionic acid became contaminated with a species of Penicillium, which grew luxuriantly in these solutions. This was not the case with the sulphuric acid solutions.

The toxicity of the salts of pentathionic acid

Although, according to Williams and Young, free pentathionic acid exhibits marked toxicity, these authors state that the neutral salts are nontoxic (52, p. 361). Accordingly, a study has been made of the toxicity of potassium pentathionate to the conidia of *Sclerotina americana,* in distilled water, in an acid buffer solution, and in a solution to which sufficient sodium hydroxide was added to cause incipient decomposition of the potassium pentathionate. The buffer used was the phthalate—NaOH buffer of Clark and Lubs (8) pH 4.6 diluted to $\frac{1}{4}$ the recommended strength. The results are shown in tables 9 and 10.

Potassium pentathionate in distilled water was entirely nontoxic at the concentration employed. When the salt is placed in an acid buffer a certain amount of free pentathionic acid must be formed, but, even under

TABLE 8.—*The toxicity of hydrogen sulphide to the conidia of Botrytis sp. (cinerea type), Macrosporium sarcinaeforme, Sclerotinia americana and to the uredospores of Uromyces caryophyllinus*

Concentration (millimols per litre solution)	*Botrytis* sp. (*cinerea* type)		*Macrosporium sarcinaeforme*		*Sclerotinia americana*		*Uromyces caryophyllinus*	
	Percentage of germination	Germ-tube length (μ)	Percentage of germination	Germ-tube length (μ)	Percentage of germination	Germ-tube length (μ)	Percentage of germination	Germ-tube length (μ)
Control	99.2	200	99.4	400	98.8	400	84.2	400
0.0002	—	—	—	—	—	—	66.8	75
0.0025	—	—	—	—	67.4	150	6.4	30
0.0250	99.4	225	24.1	100	40.5	100	1.3	15
0.0840	—	—	—	—	8.5	50	—	—
0.2500	89.5	175	2.6	50	1.5	25	0	0
0.4600	—	—	—	—	—	—	—	—
0.8300	27.8	75	—	—	—	—	—	—
1.6500	7.2	50	—	—	—	—	—	—
2.4600	0.1	15	0	0	—	—	0	0

TABLE 9.—*The toxicity of potassium pentathionate solutions to the conidia of Sclerotinia americana*

Solution	Percentage of germination	Germ-tube length (μ)
Control	99.2	500
0.10% $K_2S_5O_6 \cdot 1\frac{1}{2}\ H_2O$ in redistilled water	99.4	500
0.01% $K_2S_5O_6 \cdot 1\frac{1}{2}\ H_2O$ in potassium acid phthalate—NaOH buffer, pH 4.6	99.4	500
0.10% $K_2S_5O_6 \cdot 1\frac{1}{2}\ H_2O$ in potassium acid phthalate—NaOH buffer, pH 4.6	99.1	500
0.50% $K_2S_5O_6 \cdot 1\frac{1}{2}\ H_2O$ in potassium acid phthalate—NaOH buffer, pH 4.6	99.2	500
Potassium acid phthalate—NaOH buffer, pH 4.6	99.4	500

TABLE 10.—*The toxicity, to the conidia of Sclerotinia americana, of colloidal sulphur formed from solutions of potassium pentathionate and sodium hydroxide*

Solution	pH	Percentage of germination	Germ-tube length (μ)
Control	—	98.5	275
95 c.c. 0.5% $K_2S_5O_6 \cdot 1\frac{1}{2}\ H_2O$ + 5 c.c. N/10 NaOH	6.72	0	0
ditto, diluted 1–10	6.40	62.5	75
5 c.c. N/10 in 100 c.c. redistilled H_2O	11.14	83.9	200
ditto, diluted 1–10	10.49	97.6	350

these conditions, no toxicity was exhibited, although the pH value of 4.6 lies within the range of maximum toxicity according to Young (55, p. 410).

In the case of the solutions to which sodium hydroxide was added, due to partial decomposition, colloidal sulphur was formed, and these solutions were highly toxic.

THE TOXICITY OF WATER EXTRACTS FROM SULPHUR

Williams and Young (52, p. 359) have stated that pentathionic acid is found in filtered water extracts from sulphur. In another place (57, p. 19) the same authors say that this acid is adsorbed quite completely by the sulphur particle so that none can be washed off. According to our experience most samples of sulphur give water extracts which respond to tests given by pentathionic acid. (a) They form a brownish black precipitate of silver sulphide with ammoniacal silver nitrate. (b) When hydrogen sulphide is passed through the extracts for several minutes, colloidal sulphur is formed. (c) There is a slight precipitate formed when the extracts are made alkaline with sodium hydroxide. None of the extracts

examined gave the methylene blue test for hydrogen sulphide. The reactions obtained were, however, not very distinct, and other substances than pentathionic acid might have given each of them; we are only justified in saying, therefore, that there is a strong probability of the existence of traces of pentathionic acid in the water extracts but not an absolute certainty.

The toxicity of water extracts from several samples of sulphur has been determined. These extracts were made by triturating 100 gram portions of sulphur in a mortar with 100 c.c. of distilled water and filtering the mixture. Three of the sulphur samples were obtained from a well-known firm dealing in chemical reagents and correspond to the sulphur preparations listed in the U. S. Pharmacopeia and others were two well-known brands of commercial 300-mesh dusting sulphur. One of the latter gave an alkaline water extract which showed the presence of calcium when tested with ammonia and ammonium oxalate. None of the extracts tested showed any toxicity to conidia of *Sclerotinia americana* when prepared as described above. The results appear in table 11.

TABLE 11.—*The toxicity of water extracts from various kinds of sulphur to the conidia of Sclerotinia americana*

Solution	pH	Percentage of germination	Germ-tube length (μ)
Control	——	99.3	900
Water extract from Sulphur Lotum	6.0	99.5	900
" " " Sulphur Praecipitum	6.4	99.4	800
" " " Roll Sulphur	6.2	99.3	900
Control	——	97.6	175
Water extract from commercial dusting sulphur (A)	4.2	98.2	180
Water extract from commercial dusting sulphur (B)	8.6	98.8	225

THE TOXICITY OF SULPHUR DUST BEFORE AND AFTER TREATMENT TO REMOVE ACIDS

When it is desired to compare two samples of sulphur and to determine the effect of some factor on their toxicity, as, for example, the presence or absence of pentathionic acid, it is necessary that the samples should be alike in other respects. One factor that might be expected to influence the toxicity is the particle size of the material. Roll sulphur was ground and sieved to furnish four samples whose particles varied from 33 to 285 μ in diameter. The average diameter for each lot was determined by micro-

metric measurement of the particles. The toxicity to conidia of *Sclerotinia americana* was determined in quadruplicate tests on each sample. The results are shown in table 12.

TABLE 12.—*The relation between the particle size and toxicity of a sulphur dust to the conidia of Sclerotinia americana*

Treatment	Mean diameter of particle (μ)	Percentage of germination
Control	—	97.6
Ground Roll Sulphur	285	62.8
" " "	142	47.2
" " "	60	29.1
" " "	33	20.7

It will be seen that the toxicity increases to a marked degree as the particles decrease in size, and therefore particle size must be considered in the evaluation of sulphur dusts as fungicides.

If the toxic factor of sulphur were pentathionic acid, it would be reasonable to expect a difference in toxicity between sulphur, treated in such a way as to destroy any traces which might exist, and the original sample. Accordingly, a sample of 300-mesh dusting sulphur which gave a marked test with ammoniacal silver nitrate, was divided into two portions. One of these was triturated in a mortar with one per cent sodium hydroxide solution and allowed to remain in contact with the solution overnight. The next morning it was filtered with suction and thoroughly washed until the washings were neutral to litmus. Just before use, a portion was extracted with distilled water and the extract tested for pentathionic acid with ammoniacal silver nitrate. None was found. Comparative toxicity tests, using the conidia of *Sclerotinia americana,* were then made on the two samples in triplicate, 5000 spores being counted. The results of this experiment are shown in table 13.

TABLE 13.—*The comparative toxicity, to Sclerotinia americana conidia, of sulphur dust before and after treatment to remove pentathionic acid*

Treatment	Percentage of germination	Germ-tube length (μ)
Control	98.0	400
300-mesh sulphur untreated; with original pentathionic acid content	64.7	100
300-mesh sulphur treated to remove content of pentathionic acid	64.4	100

There is no significant difference in toxicity between sulphur treated to remove pentathionic acid and untreated sulphur.

SUMMARY

1. The various theories that have been advanced to account for the fungicidal action of sulphur have been briefly reviewed, with especial emphasis on the pentathionic acid hypothesis of Young.

2. An improved technique has been employed for the laboratory determination of fungicidal activity, by means of spore-germination tests.

3. The spores of four representative pathogenic fungi were used, namely, *Botrytis* sp. (*cinerea* type), *Macrosporium sarcinaeforme*, *Sclerotinia americana*, and *Uromyces caryophyllinus;* these exhibit varying degrees of sulphur sensitivity.

4. The accuracy attained in these tests has been determined, and defined in terms of percentage of germination and odds of significance.

5. The chemistry of pentathionic acid has been discussed, and the preparation, analysis, and properties of potassium and barium penathionates have been described. Pentathionic acid solutions have been prepared from these salts.

6. The comparative toxicity of pentathionic acid, sulphuric acid, and hydrogen sulphide to the four fungi has been determined. It has been found that pentathionic and sulphuric acids, both typical strong mineral acids, exhibit identical toxicity within the error of the experiment. The toxicity of these acids is apparently due to the hydrogen ion, and a comparatively high concentration is required for its manifestation. Hydrogen sulphide is from 6 to 200 times as toxic.

7. When percentage of germination is plotted against concentration of a toxic agent, a sigmoid curve is obtained. This curve appears to be the integrated form of a normal distribution curve, which perhaps indicates the distribution of resistance among the individual spores used.

8. The neutral salts of pentathionic acid were found to be nontoxic to conidia of *Sclerotinia americana,* except when treated with sodium hydroxide, which destroys the pentathionic and forms colloidal sulphur.

9. It has been found that most samples of sulphur give water extracts that respond to qualitative tests for pentathionic acid, but these extracts were not toxic under the conditions of experiment.

10. The particle size of sulphur dusts is an important factor in their toxicity and must be considered in comparing one preparation with another. The toxicity increases with the fineness of subdivision.

11. A commercial 300-mesh dusting sulphur, treated with sodium hydroxide to remove pentathionic and sulphuric acids, did not differ in toxicity from the same preparation before treatment.

12. It is therefore concluded that pentathionic acid is not a factor of importance in the fungicidal action of sulphur.

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EFFECT ON PLANTS OF CYANIDE FUMIGATION FOLLOWING SPRAYING WITH BORDEAUX MIXTURE

O. Butler and R. R. Jenkins

Woglum[1] has on several occasions called attention to the danger of cyaniding citrus trees sprayed with Bordeaux mixture. The lower branches of the trees are sometimes sprayed with Bordeaux mixture and the fungicide in paste form is also applied to the trunk and branches. When the mixture is applied to the trunk the trees can be fumigated immediately without injury, but if it is applied to the branches, also, or to the branches only, or the tree has been sprayed, then fumigation can not safely be given until six months have elapsed. Injury has been observed even when fumigation was delayed eleven months. Woglum suggests that the injury produced by cyanide fumigation following an application of Bordeaux mixture is due to the formation of a copper cyanide.

Guba[2] has found that cucumber and tomato plants sprayed with Pyrox or Bordeaux mixture or dusted with copper-lime dust and then subjected to cyanide fumigation are injured. He ascribes the injury to the formation of cupric cyanide, an unstable salt decomposing with liberation of cyanogen to form cuprous cyanide, and concludes that plants exposed to hydrocyanic-acid gas should never be sprayed antecedently with a copper fungicide.

The Bordeaux mixture used in citrus spraying is alkaline; the mixtures and copper-lime dust employed by Guba are alkaline. It has seemed to us desirable, therefore, to study the effect of the ratio copper sulphate to quick lime, used in preparing a Bordeaux mixture, on the injuriousness of cyanide fumigation, and to subject to experimental verification the suggestion of Guba that hydrocyanic-acid gas combines with the copper to form unstable cupric cyanide. We shall here present successively: (1) The effect of ratio copper sulphate to quick lime on toxicity of hydrocyanic-acid gas; (2) the effect of wetting foliage after cyanide fumigation; and (3) the chemical changes involved when Bordeaux mixtures of various types react with hydrocyanic-acid gas.

I

In our experiments we used sodium cyanide at the rate of 0.20 oz. per 1000 cubic feet, the hydrocyanic-acid gas being generated by mixing the

[1] Woglum, R. S. Fumigation of citrus trees. U. S. Dept. Agr. Farmers' Bul. 923. 1918. Fumigation of citrus plants with hydrocyanic acid: Conditions influencing injury. U. S. Dept. Agr. Bul. 907. 1920. Fumigation of citrus trees for control of insect pests. U. S. Dept. Agr. Farmers' Bul. 1321. 1923.

[2] Guba, E. F. Injury to glass-house plants from hydrocyanic acid gas following the application of copper fungicides. Phytopath. 16: 633–634. 1926.

TABLE 1.—*Effect of composition of Bordeaux mixture on injury to plants following cyanide fumigation*

Subjected plant	No. of experiments	Plants not sprayed before cyaniding	Extent of injury following fumigation. Plants sprayed before cyaniding with Bordeaux mixture						
			1: 0.2	1: 0.5	1: 1	1: 2	1: 4	1: 6	Milk of lime
Sunflower	5	none	trace	v. slight	bad	slight	trace	trace	trace
Tomato	5	trace	v. slight	slight	v. marked	marked	slight	v. v. slight	trace
Bean	4	none	slight	slight	bad	marked	slight	v. slight	v. slight
Cucumber	4	none	trace	v. v. slight	v. bad	slight	trace	none	none
Castor-oil plant	4	none	trace	trace	slight	v. slight	none	none	none
Potato	3	none	none	trace	marked	marked	v. v. slight	trace	none

sodium cyanide, concentrated sulphuric acid, and water in the following proportions:

Sodium cyanide, 2 parts; sulphuric acid conc. C. P., 1½ parts; water, 2 parts.

The plants were cyanided for approximately 12 hours, *i.e.*, from one hour after sundown to daybreak and at a mean temperature of approximately 18° C. The plants in each series of an experiment were sprayed in triplicate and arranged on the benches in sequence so as to obtain as nearly as possible equal exposure to the hydrocyanic-acid gas. The sprays were applied the same day the plants were cyanided but sufficiently early to assure the foliage being dry before sundown. The Bordeaux mixtures contained 1 per cent copper sulphate and were prepared in the following ratios of cupric sulphate to calcic oxide 1:0.2, 1:0.5, 1:1, 1:2, 1:4, and 1:6. The mixture prepared in the ratio 1:0.2 was made with limewater and the requisite strength in copper sulphate obtained by allowing the mixture to settle and decanting. The other mixtures were made with milk of lime of the required strength. Besides the Bordeaux mixtures, milk of lime containing 1 per cent of calcic oxide was used.

The results obtained are summarized in table 1.

The data presented in the table show unmistakably that the amount of lime present in the Bordeaux mixture determines to a very marked degree the resultant injury. As the ratio of copper sulphate to quick lime is raised injury increases and then decreases for all the plants employed in the experiment. The data show that a 1:1 mixture, which is the one most frequently used in the United States, is, in general, the most injurious. In the case of the potato, however, in two out of the three experiments performed the 1:2 mixture proved more injurious. In the case of the bean the mixtures of the two ratios were equally injurious once; in the case of the tomato the 1:2 mixture was more injurious in one fifth of the experiments; but, for the other plants, the 1:2 mixture was never more injurious than the 1:1 mixture. Attention should be drawn also to the fact that the change in relative position of plants sprayed with Bordeaux mixture 1:1 occurs only in those instances where the injury produced by each type of mixture approximates rather closely that of the other as a glance at the data presented in table 2 will show, but has nothing to do with the sensitivity of the plant, since the castor bean, the most resistant of those employed, shows in round numbers twice the injury when a 1:1 mixture is used than it does when a 1:2 mixture is applied, and the cucumber, extremely sensitive to cyanide-fumigation following spraying, exhibits a like effect. To consider further the data presented in table 1, it will be noted that plants sprayed with neutral Bordeaux mixture and the mixture highest in lime suffer little injury when cyanided, the former being somewhat more

TABLE 2.—*Relative injuriousness of cyanide fumigation to plants sprayed with Bordeaux mixtures containing copper sulphate and quick lime in the ratios of 1:1 and 1:2, respectively*

Subjected plant	No. times 1:2 mixture proved more injurious than 1:1 mixture	Relative percentage of injury caused by	
		1:1 mixture	1:2 mixture
Sunflower	0	100	58
Bean	$\frac{1}{4}$	100	77
Cucumber	0	100	47
Tomato	$\frac{1}{5}$	100	84
Castor-oil plant	0	100	54
Potato	$\frac{2}{3}$	100	85

injurious. Plants sprayed with milk of lime show no more injury following cyanide fumigation than do the nonsprayed plants. Lime, in and of itself, therefore, is not injurious.

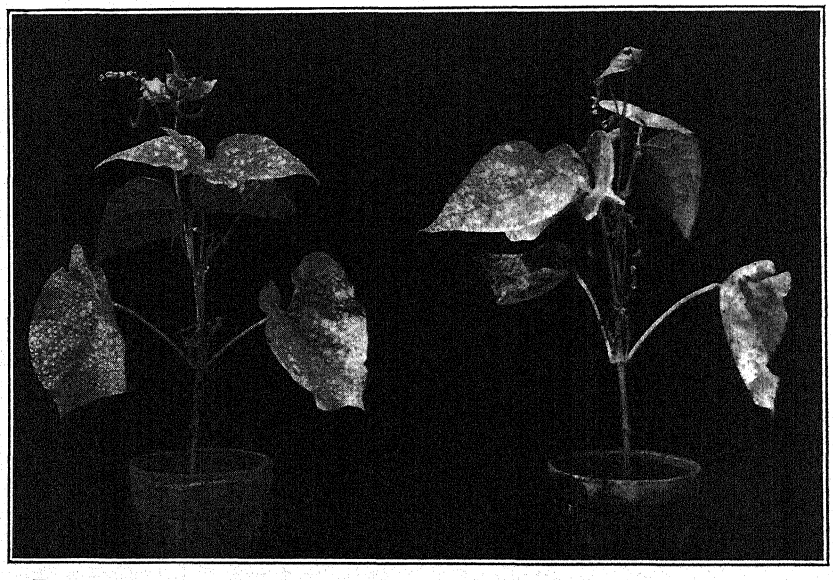


FIG. 1. Bean plants sprayed with 1 per cent Bordeaux mixture 1:1, afternoon of May 17, and cyanided from sunset the same day to sunrise May 18. During the late afternoon of May 18 the foliage of the plant on the right of the figure was sprayed with distilled water and placed in a moist chamber over night. Photographed May 20. The apical leaves of the plant on the left are badly scorched and one cotyledonary leaf shows peripheral scorching. The plant on the right shows more serious injury, both cotyledonary leaves are badly scorched and curled, and the apical leaves are also seriously injured. Wetting the foliage has noticeably increased the injury due to cyanide fumigation.

It will also be observed that neutral Bordeaux mixture causes but little more injury than milk of lime. We may conclude, therefore, that since the copper precipitate in a neutral mixture has the same composition as the precipitate in freshly made alkaline washes, basic-copper sulphate, in and of itself, is not injurious. To produce injury an excess of calcium hydrate is required. But, in the case of the alkaline Bordeaux mixtures employed by the writers, the mother liquors give identical reactions; hence, it may be further concluded that the presence of insoluble calcium hydroxide in definite proportion to copper sulphate determines the degree of injury following cyanide fumigation.

II

The effect of cyanide fumigation following spraying with the Bordeaux mixtures described in the previous section is materially modified by the after treatment given the plants. When the foliage of sprayed and cyanided plants is wetted with water a day or so after the exposure to hydrocyanic-acid gas and is kept moist over night, the injury originally produced by 1:0.2, 1:0.5, and 1:1 mixtures is not markedly changed, but the 1:4 and 1:6 mixtures show extreme toxicity. In figures 1 to 6 is shown the effect

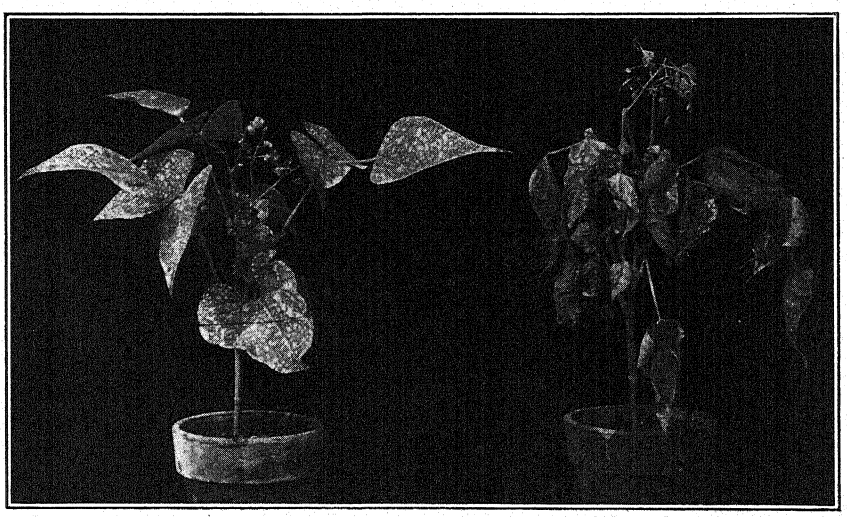


FIG. 2. Bean plants sprayed with 1 per cent Bordeaux mixture 1:6 but otherwise given same treatment as those illustrated in figure 1. The plant shown on the left is uninjured; the one on the right, the foliage of which was wetted, is dead.

produced by wetting the foliage of bean and cucumber plants previously sprayed with Bordeaux mixture 1:1 and 1:4 or 1:6, and cyanided.

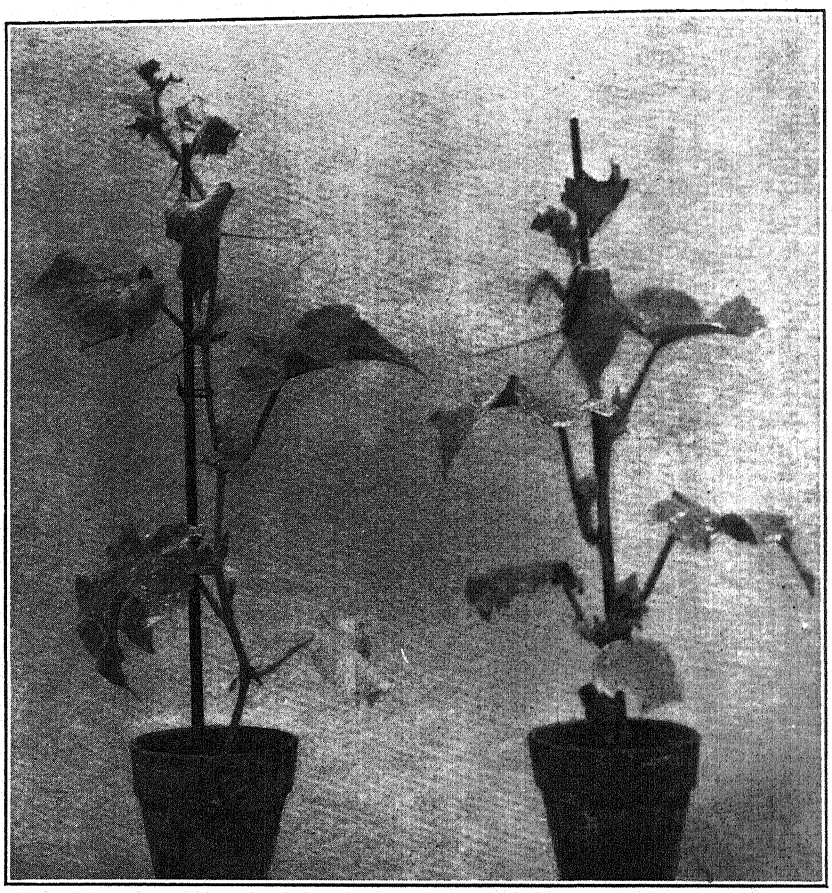


FIG. 3. Cucumber plants sprayed with 1 per cent Bordeaux mixture 1:1 and cyanided. The younger leaves are scorched. Compare with figure 4.

In table 3 are presented the data obtained in an experiment designed to show the effect produced by wetting the foliage of sprayed and cyanided plants. On the late afternoon of the day following cyanide fumigation, the plants were divided into two sets. In one of the sets the foliage of the plants was allowed to remain dry; in the other, it was wetted and kept moist over night.

The data presented show clearly that Bordeaux mixtures high in lime can not be applied to plants subject to a cyanide-fumigation régime unless means are provided to keep the foliage dry at all times. Bordeaux mixtures prepared with copper sulphate-quick lime ratios of 1:4 and 1:6, and which are so poisonous when wetted following cyanide fumigation, presumably retain their toxic properties indefinitely. On the other hand, a neutral

TABLE 3.—*Effect of composition of Bordeaux mixture on the injury produced by wetting with water following cyanide fumigation*

Subjected plant	Treatment of foliage	Injury to plants sprayed before cyaniding with Bordeaux mixtures in the following ratios:			
		1: 0.2	1: 1	1: 4	1: 6
Cucumber	not wetted	none	marked	none	none
	wetted	slight	marked	very bad	very bad
Bean	not wetted	v. slight	bad	none	none
	wetted	slight	bad	very bad	very bad
Buckwheat	not wetted	none	none	none	none
	wetted	none	v. slight	very bad	very bad
Sunflower	not wetted	none	marked	none	none
	wetted	v. slight	marked	very bad	very bad

Bordeaux mixture may be used with safety and, judging from an experiment made with tomatoes, we presume that, in practice, Bordeaux mixtures in which the copper sulphate-quick lime ratio was 1: $\frac{1}{4}$ could be safely employed.

III

When hydrocyanic acid gas is passed into a Bordeaux mixture the nature of the resultant precipitate depends on the ratio of cupric sulphate to calcic oxide in the mixture. The color of the basic copper sulphate is rapidly discharged and the changes indicated in table 4 are obtained.

TABLE 4.—*Effect of composition of Bordeaux mixture on the reaction with hydrocyanic acid gas*

Ratio of $CuSO_4 5H_2O$ to CaO	Color of solution and of precipitate	
	At beginning	After 24 hours
1: 0.2	solution, colorless precipitate, yellowish green	solution, colorless precipitate, white
1: 0.5	solution, pink precipitate, white	solution, slightly yellow precipitate, dirty white
1: 1	solution, light brown precipitate, brownish green	solution, dark brown precipitate, yellowish brown
1: 2	solution, brown precipitate, yellowish brown	solution, dark brown precipitate, reddish brown
1: 4	solution, light yellow precipitate, white	solution, light brown precipitate, yellowish brown
1: 6	solution, colorles precipitate, white	solution, colorless precipitate, white

It will be noted that those Bordeaux mixtures which are more especially toxic to plants following cyanide fumigation are those in which the resultant precipitate is a shade of brown; while for those that are not toxic, *i.e.*, mixtures in which the ratio of copper sulphate to quick lime is 1: 0.2

and 1:6, respectively, it is white. The brown precipitates, washed in several changes of distilled water and spread on glass plates, give off cyanogen;

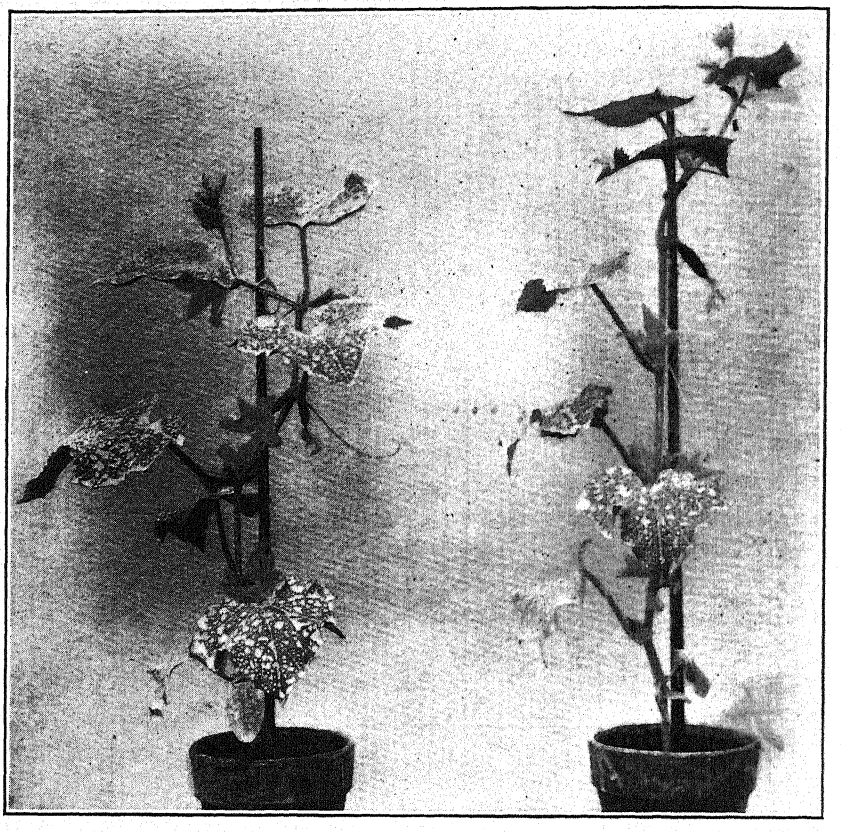


FIG. 4. Cucumber plants sprayed with 1 per cent Bordeaux mixture 1:4 and cyanided. Compare with plants shown in figure 3.

the white precipitate, washed and spread on glass plates, do not. The brown precipitates possess the properties of cupric cyanide. The white precipitates have the property of cuprous cyanide in the case of the 1:0.2 mixture, but, in the 1:6 mixture, we believe a double cyanide of copper and lime is formed, for the precipitate washed with water gives an alkaline solution containing all the copper and an abundance of lime in solution. It remains now to show that the Bordeaux mixtures that are toxic following cyanide fumigation actually combine with hydrocyanic-acid gas and gradually decompose with formation of cyanogen. This may easily be done in the following manner: The mixtures are sprayed on glass slides, dried, and exposed in the greenhouse to the cyanide fumigation given plants, *i.e.*, in

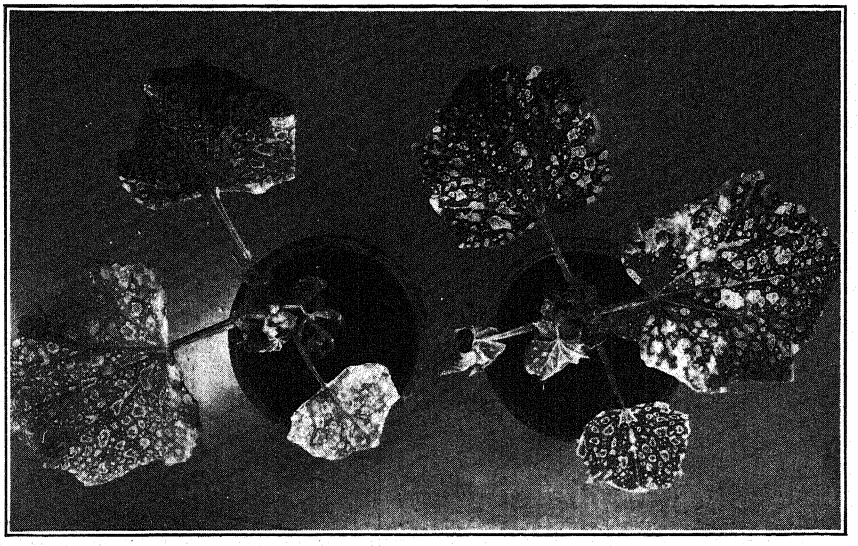


FIG. 5. Cucumber plants sprayed with 1 per cent Bordeaux mixture 1:1 and cyanided. On the afternoon of the day following fumigation the foliage of the plant on the right was wetted with distilled water and the plant placed in a moist chamber over night. The photograph was taken 24 hours later. Wetting the foliage has intensified the injury produced. Compare with figure 6.

our experiments, 0.20 oz. of sodium cyanide per 1000 cubic feet, and 12 hours exposure. The slides after cyaniding are allowed to air for a while and are then placed in petri dishes with a drop of water. Slips of filter paper are cut to the size of the glass slides, dipped in an alkaline picrate solution,[3] drained, laid on clean slides, and supported over the sprayed slides with a clearance of 2 mm. The petri dishes were then covered and allowed to stand 24 hours. After the paper was exposed for the length of time described it was rapidly dried and the colors matched to Ridgway's Standards.[4]

When a soluble cyanide is added to an alkaline picrate solution a salt of isopurpuric acid is formed which gives the solution a reddish brown color. In the presence of cyanogen the test paper gradually changed from pure yellow to shades of orange as the amount of cyanogen given off increased. In the absence of cyanogen the color of the paper was strontian yellow in the presence of increasing amounts of cyanogen the colors noted were, in order, wax yellow, primuline yellow, yellow ocher, ochraceous buff, and ochraceous orange.

[3] The solution was prepared according to the formula of R. G. Smith. A method for the quantitative determination of cyanide in small amounts. Jour. Amer. Chem. Soc. 51: 1171–1174. 1929.

[4] Ridgway, R. Color standards and color nomenclature. Washington, 1912.

TABLE 5.—*Effect of composition of Bordeaux mixture on the discharge of cyanogen following fumigation with hydrocyanic acid gas*

Mixture	Color of alkaline picrate after an exposure of		
	6 hours	12 hours	24 hours
Bordeaux 1: 0.2	strontian yellow	strontian yellow	strontian yellow
do 1: 0.5	strontian yellow	strontian yellow	primuline yellow
do 1: 1	yellow ocher	yellow ocher	yellow ocher
do 1: 2	wax yellow	primuline yellow	ochraceous orange
do 1: 4	strontian yellow	strontian yellow	ochraceous buff
do 1: 6	strontian yellow	strontian yellow	strontian yellow
Calcium hydrate 1% CaO	strontian yellow	strontian yellow	strontian yellow
Witness	strontian yellow	strontian yellow	strontian yellow

The results of one of the experiments are presented in table 5 and show conclusively that Bordeaux mixture made with a 1: 1 ratio of copper sulphate to quick lime gives off cyanogen in largest amount and most rapidly. In the case of the 1: 2 mixture the evolution of cyanogen is much slower than in the 1: 1 mixture; in fact, after the lapse of 24 hours, a 1: 2 Bordeaux mixture has given off only as much cyanogen as a 1: 1 mixture in 6 hours. Bordeaux mixtures in which the ratio of copper sulphate to quick lime is 1: 4 and 1: 0.5, respectively, evolve cyanogen very slowly; but neither a 1: 0.2 nor a 1: 6 mixture, or milk of lime, gives off detectable amounts. Now, since calcium cyanide decomposes in moist air, it is clear that this salt does not form. It is also evident that cupric cyanide does not form in either a 1: 0.2 or 1: 6 Bordeaux mixture. We have already shown that a 1: 0.2 Bordeaux mixture is not toxic following cyanide fumigation when the foliage is wetted and does not therefore contain a soluble cyanide. On the other hand, Bordeaux mixture in which the ratios of copper sulphate to quick lime are 1: 1, 1: 2, 1: 4, and 1: 6 contains soluble cyanide in increasing amounts, and when the foliage of plants sprayed with these mixtures and fumigated is wetted the washes highest in lime cause death; the others result in increased injury. (Figs. 1, 2, 5, 6.)

SUMMARY

1. The formation of cupric cyanide in a Bordeaux mixture exposed to cyanide fumigation is determined by the ratio of copper sulphate to quick

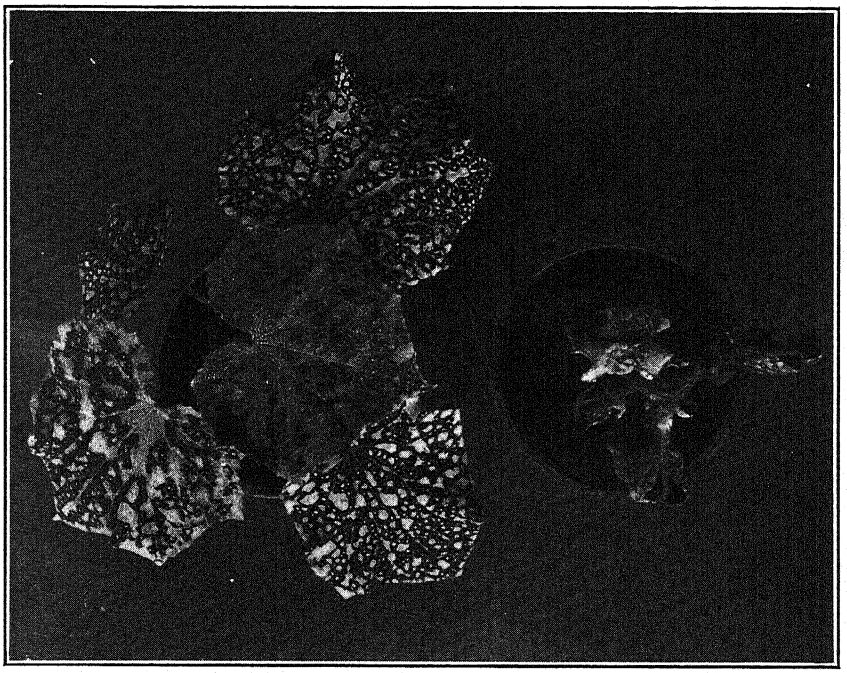


FIG. 6. Cucumber plants sprayed with 1 per cent Bordeaux mixture 1: 6 and cyanided. On the afternoon of the day following fumigation the foliage of the plant on the right was wetted with distilled water and the plant placed in a moist chamber with the plant similarly treated and shown in figure 5. The photograph was taken 24 hours later. The plant on the left, the foliage of which was kept dry following fumigation, is uninjured; the plant on the right, the foliage of which was wetted, is dead. Compare with figure 5.

lime used in making the mixture. Cupric cyanide does not form when the ratio employed is 1: 0.2 and in negligible amounts only when the ratio is 1: 4, or higher.

2. In a 1: 0.2 mixture insoluble cuprous cyanide is formed; in a 1: 6 mixture a soluble double cyanide. In mixtures of ratios greater than 1: 1 but less than 1: 6 the amount of cupric cyanide formed decreases with increase in calcium hydrate, and the double cyanide becomes more abundant.

3. A Bordeaux mixture, forming with hydrocyanic acid gas a double cyanide, in injurious if the sprayed and cyanided plants are wetted.

4. A neutral Bordeaux mixture, or an approximately neutral mixture, is the only type to be recommended when the sprayed plants are to be subjected to cyanide fumigation. It should then be used only on plants that are no more sensitive to soluble copper than is the tomato.

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THE RELATION OF PROTOCATECHUIC ACID TO DISEASE RESISTANCE IN THE ONION[1]

H. R. Angell, J. C. Walker, and Karl Paul Link

INTRODUCTION

Relatively greater resistance to certain diseases has long been known in those varieties of onion in which red or yellow pigment is produced in the bulb scales as they approach maturity. It is especially noticeable in the case of smudge, caused by *Colletotrichum circinans* (Berk.) Vogl., and neck rots caused by *Botrytis* spp. The nature of this resistance was first studied by Walker (5, 6). It was found that in the case of the colored onions a water-soluble toxic substance (or substances) occurred in the dry outer scales of the bulbs. In this toxic solution the growth of the smudge organism was checked, the tips of young germ tubes commonly ruptured, or the spores were killed without germinating. It was thus interpreted that during periods when moisture was present upon the bulbs and when infection by the smudge organism ordinarily occurred the toxic substance (or substances) present in the tissue diffused into the water and precluded further advance by the fungus, if it were present. No such toxic effects were found in the dry outer scales of white bulbs, which were very susceptible to smudge.

Further studies (8, 9) showed that the extracts of outer colored onion scales were toxic not only to the smudge and neck-rot organisms but also to several other fungi tested, some of which were pathogenes of the onion and others were not. *Aspergillus niger* was the only organism tested the growth or germination of which was not greatly inhibited. Correlation of resistance with the production of red or yellow pigments was found only in the case of those organisms which normally penetrate the onion in such a way as to encounter the dry outer scales before entry. Thus, in the case of Fusarium bulb rot, where the organism enters by way of the root scars or of the basal tissue of the bulb, no resistance was noted, even though the parasite was inhibited when exposed to the scale extract.

It was further shown that the toxic substance is readily soluble only from the dry outer scales and that it functions as a resistant property of these scales only as it diffuses from them. The inner fleshy scales, even though colored, are readily attacked by the smudge fungus when the

[1] This study is the result of cooperative investigations between the Department of Plant Pathology, University of Wisconsin, and the Office of Horticultural Crops and Diseases, Bureau of Plant Industry. The initial chemical investigations were performed by Angell. The final steps in the isolation and identification of the toxic compound were carried out by Link.

inoculum is applied directly to them. The reason for this is not known, but two possible explanations are offered. It may be readily demonstrated that as the fungus penetrates the cuticle of the fleshy scales the pigment which is confined to the epidermal cell rapidly disappears, apparently before the hyphae have traversed the subcuticular wall to enter the cell lumen. May the toxic substance not also be destroyed with the pigment? Another possibility, still unsettled, is whether the pigment and the toxic substance are identical. If they are not the same, is the toxic substance absent from the fleshy scales, and does it appear only in the outer scales after they have dried? In any case, it is certain that the outer dry scales containing the readily diffusible toxic principle serve as a protective resistant barrier against the smudge organism, but in white onions the uncolored outer scales have no such property. This paper is a report upon the results of an investigation which has led to the conclusion that a phenolic acid, known as protocatechuic acid, is one of the substances responsible for the resistant character of colored onion varieties.

EXPERIMENTAL RESULTS

Methods. Quantities of the dry outer scales of white, red, and yellow onions were secured from the bulbs after harvest. From such materials extractions were made by the use of various solvents. The various steps taken in the fractionation of the extracts are described in detail during the course of the paper. Standard color and precipitation tests for the identification of phenolic substances were used. Since these tests are fully discussed by Haas and Hill (1) it seems unnecessary to repeat their description here.

During the steps of the extractions the fractions were tested repeatedly for their toxicity to the spores of *Colletotrichum circinans*. The rupturing of the tips of the germ tubes, characteristic of the crude toxic solutions, already described (6), was relied upon as an indicator as to whether the unknown toxic substance was being preserved during the course of the extraction.

Preliminary steps. Preliminary qualitative tests made with cold-water extracts from dead outer scales of white, yellow, and red onions indicated the presence of soluble phenolic compounds in the solutions from colored scales and their absence from those from white scales. Perkin and Hummel (4) had previously shown the presence of free quercetin, a phenolic compound, in colored scales of onion, but, since this substance is quite insoluble in cold water, it could not be the cause of the positive reaction for phenols nor could it be responsible for the toxicity of such solutions. The latter statement is further substantiated by the fact that crystals of quercetin, prepared from colored onion, when added to a suspension of spores of *C.*

circinans had no effect upon germination and growth. As pointed out in a previous paper (2), the possibilities of a soluble glucoside of quercetin and of a tannin glucoside also were excluded. The next problem, therefore, was to separate, if possible, the toxic compound or compounds from impurities and at the same time retain their original chemical structure and toxic character.

After preliminary tests of the efficacy of ether, 95 per cent alcohol, ethyl acetate, and acetone for the removal of the toxic substance by continuous extraction, the last-named solvent was selected as being the most suitable. Yellow scales were dried at 80° C., finely ground, and continuously extracted with acetone for 14 hours. The extract contained the toxic substance together with quercetin, traces of glucose, fat-like substances, and a reddish brown pigment of an unknown nature. In order to get rid of the quercetin, the acetone was evaporated, and water, in which quercetin is insoluble, was added to the dry residue. The toxic substance and the water-soluble impurities went into solution, which upon filtering, gave the typical qualitative reaction of the ordinary aqueous extract of the scales. When tested against spores of *C. circinans,* it completely prevented germination in dilutions of 1 to 8 and 1 to 12. At 1 to 16 an occasional spore started to germinate but the growing tips ruptured, while the latter reaction was more general at 1 to 20 and very abundant at 1 to 30. No normal germination occurred except at 1 to 30 when an occasional short germ tube was noted. This procedure was repeated several times with similar results.

This crude aqueous solution was next concentrated to a thick sirup under reduced pressure at temperatures below 40° C. The concentrate was treated with a mixture consisting of about 20 times its volume of alcohol and 40 times its volume of ether and allowed to stand for some hours. The reddish brown precipitate was filtered, dried, and brought into solution in water. It gave only a very faint reaction with ferric chloride and, when tested against spores of the smudge organism, it prevented germination at a dilution of 1 to 2 but had no inhibitive effect at 1 to 5. It was obvious that only a trace of the toxic substance was present in the precipitate.

The alcohol-ether solution was evaporated, and a portion of the residue, which was of a light-orange color, was taken up in water. It gave a strong ferric-chloride reaction. When tested with spores of *C. circinans* it entirely prevented germination at dilutions up to 1 to 120. At dilution 1 to 240 and 1 to 480 a large percentage of the spores had ruptured germ tubes and no normal germ tubes were present. At 1 to 960 a small percentage of the spores formed germ tubes but growth was distinctly retarded and an occasional tube ruptured. At 1 to 1920 a high percentage of the spores

germinated and normal growth ensued without any evidence of inhibitive effect.

In another experiment the residue from an acetone extract was dried and then taken up in water and filtered. The aqueous solution was shaken with ethyl acetate until separation was as complete as practicable. The ethyl acetate was evaporated, and part of the almost colorless residue was dissolved in water. It gave a very strong ferric-chloride reaction when tested against spores of the smudge organism. The solution was toxic in dilutions up to 1 to 3300 in which rupturing of the germ tubes was common but no normal germ tubes were noted. At lower dilutions no germination whatsoever occurred. At 1 to 6600 there was a high percentage of normal germ tubes and an occasional ruptured one.

Extracted scales not toxic. After the acetone extraction, samples of the extracted scales were dried and tested for toxicity. Suspensions of spores of *C. circinans* in drops of water were added to suitable amounts of the scales on glass slides, which were then placed in moist chambers and left for 24 hours. Checks with ground unextracted scales were also made. After 24 hours the spores in the drop of water around the extracted scales had all germinated and produced normal germ tubes, whereas rupturing of germ tubes was general among those spores in the solution containing the unextracted scales.

This showed that all of the toxic material was removed and carried into the acetone solvent.

Isolation of and toxicity tests with protocatechuic acid. In the experiments thus far reported it was shown that the toxic principle was completely removed from colored scales by extraction of the latter with acetone and that it could be separated from a large proportion of the nontoxic impurities by the use of neutral solvents at temperatures not exceeding the boiling point of acetone (57° C.). Attempts were next made to obtain the toxic principle in larger quantity. A somewhat modified method of extraction was followed, in which the initial extraction was made in water instead of in acetone. Three bags of yellow-onion scales weighing about five pounds each were steeped overnight in sufficient water to cover them. The colored aqueous extract was treated first with neutral and then with basic lead acetate until no more precipitate was obtained. The latter was filtered off, suspended in water, and decomposed with 2.5 per cent sulphuric acid. The solution was then shaken with ether, the ether solution was separated, and the solvent was evaporated. The residue contained a mixture of isolated crystals and an amorphous pigmented substance, which when dissolved in water gave a strong ferric-chloride test. The solution was toxic up to a dilution of one part of the dried residue in 1900 parts of water, in which a large percentage of the spores showed ruptured germ tubes after 24 hours.

Another lot of scales was then treated in a similar manner and an effort made to obtain the crystals in pure form. The details of their purification and identification as protocatechuic acid (3, 4-dihydroxybenzoic acid, $C_7H_6O_4$) are reported elsewhere (2, 3). When the pure acid was finally obtained it was dissolved in water and tested against spores of *C. circinans* in a number of dilutions. At 1 to 100, 1 to 200, 1 to 400, and 1 to 800 it prevented germination of the spores. At 1 to 1600 a high percentage of the spores germinated but practically all of the germ tubes ruptured. At 1 to 3200 the percentage of normal germ tubes was greater but still many of the germ tubes ruptured. Only at a dilution of 1 to 6400 were satisfactory germination and growth obtained.

In the results thus far reported, yellow-onion scales were used as the source of the toxic substance, protocatechuic acid. Since red varieties of onion are equally resistant to smudge, the procedure was repeated, starting with dry outer scales from bulbs of this color. From these were secured crystals of the acid identical with those of the yellow scales in composition and toxicity. All attempts to isolate the acid from white scales failed.

DISCUSSION

Wide variations in resistance and susceptibility to certain diseases has been commonly noted among varieties, strains, or individuals of many species of plants. In the cultivated species much of economic value has been and is being brought forth in the way of plant improvement through the discovery of resistant strains or through the perfection of new strains by means of selection and hybridization. The study of the nature of these differences in resistance has offered a peculiar challenge to plant scientists not only because of its purely scientific interest but also because of the obvious value of the results of such study as a basis of further plant improvement.

It is by no means implied that a common explanation may be expected for all cases of resistance in plants. Much evidence has already been accumulated to show a variety of reactions between resistant hosts and their would-be parasites. Since much of the literature pertaining to this question has been summarized elsewhere (7), it will not be discussed in detail here. In many of the cases studied one is led to suspect that the fundamental fact underlying resistance may be the presence of a substance or a group of substances in the cells of the resistant host which are toxic to the invading parasite, while in the cells of plants in a susceptible but otherwise quite similar variety these toxins are absent or so limited in quantity as to be ineffective. Such toxic substances, though present, are not usually readily isolated in their natural form, and the difficulties involved in the biochemical studies of these questions quite naturally hinder progress.

In the case of onion resistance herein described the writers were impressed with certain facts which seemed to offer an unusually attractive line of approach to this general problem. In the first place, the toxic substance or substances readily diffused from the dry outer scales into cold water. Secondly, they caused upon the germinating spores of the parasite a peculiarly characteristic effect which might be used as a basis of identifying them if they should be secured in pure form. As already shown, these two advantages greatly facilitated the work. The fact that protocatechuic acid could be extracted through the use of neutral solvents at room temperatures (20° to 30° C.) is a strong indication that it exists as such in the plant. That the toxic substance was practically unaltered during the process is shown by the fact that when the crude extract was tested during successive steps of its purification it continued to give the same chemical reactions and to cause the characteristic rupturing of the germ tubes of *C. circinans*. There seems, therefore, little doubt that protocatechuic acid, which is absent in white onions and present in the scales of colored onions, is one of the principal causes of disease resistance noted in varieties of the latter type.

This should not be interpreted, however, as implying that protocatechuic acid is the sole cause of resistance. As already stated (2), the yield of this substance by the present method is not sufficient to account for all the toxicity of the crude extract. It is reasonable to suppose that some may have been lost during extraction and purification. On the other hand, the possible presence of other closely related compounds which may contribute a share of the resistance is not to be overlooked. These are questions still under investigation.

The close association of this phenolic acid (protocatechuic acid) with the pigment quercetin, one of the pigments of the onion, should be emphasized here. Protocatechuic acid is not a pigment. It is, however, a constituent of various flavone and anthocyan pigments. Quercetin, the 1:3:3-tetrahydroflavonol, is composed of oxalic acid, phloroglucinol, and protocatechuic acid and upon alkaline fusion breaks up into these three components. The chemical stability of quercetin is of such an order that under the conditions in which the protocatechuic acid was isolated the latter could not have arisen by decomposition of the quercetin. In the pigmented onion protocatechuic acid is apparently an accompanying substance of the pigment quercetin. In the three other cases (2) it has been shown that the pigment quercetin is accompanied by the phenolic acid (protocatechuic acid).

With regard to the bearing upon the general problem of disease resistance, these results may have some significance. So far as the writers

are aware, this is the first instance where resistance to or immunity from disease in plants or animals has been definitely shown due to a specific chemical compound produced by the host, although the existence of such toxic substances has often been suspected as the cause of such phenomena. Generalization on the basis of an isolated case is unjustifiable, and it has already been emphasized here and elsewhere that even in this instance the accessibility of the toxic substance at the critical point where invasion of the parasite may occur is quite as important as the presence of the substance. On the other hand, it is not without significance that this specific substance has been found in species of plants widely removed from the onion. Further search may reveal a more general occurrence, since the class of chemical substance to which it belongs is widely distributed in the plant kingdom.

SUMMARY

This paper is a report of a study of the chemical nature of the disease-resistant principle previously noted in colored varieties of onion and not found in white ones.

Preliminary qualitative tests showed the presence of phenolic compounds in toxic water extracts from dry outer colored scales, while none were found in the water extracts from white scales.

One of the toxic compounds was isolated in crystalline form from the crude water extract of dry outer colored scales and identified as the phenolic acid commonly known as protocatechuic acid. This exists in the colored outer scales in the free state, is readily soluble in water, and in dilutions up to about 1–3000 produces the same type of toxic effect upon spores of the smudge organisms as does the crude water extract. It is not present in the white scales. This substance is considered to be one of the compounds responsible for disease resistance in colored onions.

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DRY ROT OF CORN CAUSED BY DIPLODIA MACROSPORA EARLE

A. H. EDDINS

INTRODUCTION

One of the common diseases of corn in Florida is caused by the fungus *Diplodia macrospora* Earle (5). This organism was first reported on old weathered cornstalks at Auburn, Alabama, in the spring of 1896 (4). In 1908 Stevens and Hall (8) found that it caused an ear mold of corn in North Carolina. Since only brief notes constitute the published accounts of the disease and the fungus, the studies herein reported were undertaken to obtain further information concerning the importance and symptoms of the disease, and morphology, taxonomy, physiology, pathogenicity, and survival of the causal organism.

DISTRIBUTION AND IMPORTANCE

Dry rots of corn caused by *D. macrospora, D. zeae* (Schw.) Lév. (1, 3, 7), and *D. frumenti* Ell. and Ev. (6) are well distributed in the different corn-growing localities of Florida. However, they differ from each other in frequency of occurrence and destructiveness.

A microscopic examination of the spores borne in pycnidia on 618 stalks showed that 496 stalks were infected with *D. zeae,* 102 with *D. macrospora,* and 20 with *D. frumenti.* Occasionally stalks were found with pycnidia of two species of Diplodia on them and in a few cases the three species occurred on the same stalk. Furthermore, observations made during the seasons of 1928 and 1929 in Florida show that *D. zeae* ranks first, *D. macrospora* second, and *D. frumenti* third in causing destructive ear rots of corn.

SYMPTOMS

Stevens and Hall's (8) description of the disease caused by *D. macrospora* is brief and inadequate. In Florida, this fungus attacks the ears, husks, ear shanks, stalks, leaves, and roots of corn plants. The symptoms of the disease on these plants are as follows:

Ears, husks, and shank: Badly diseased ears are shrunken and shriveled and tightly clasped by the husks. On such ears the white mycelium of the fungus is visible on the kernels and between the kernel rows (Fig. 1). Usually the inner husks also are covered with the white mold. The kernels are lusterless in appearance and often gray, though seldom black. Pycnidia may occur on the sides and tips of kernels and on the husks. In many cases only one end of an ear is diseased, the butt end being most often involved. The mycelium of the fungus may or may not be visible on the kernels of

ears that are not severely diseased. On some ears the white mold can be seen only on the tips of kernels. On others the disease is detected only when the fungous mycelium grows out of the kernels in the germinator.

The disease on the shank is recognized by the presence of pycnidia on its surface. In some cases the mycelium may be seen growing on the surface of the shank if sufficient moisture has been retained in the crevice formed by the enclosing ear sheath.

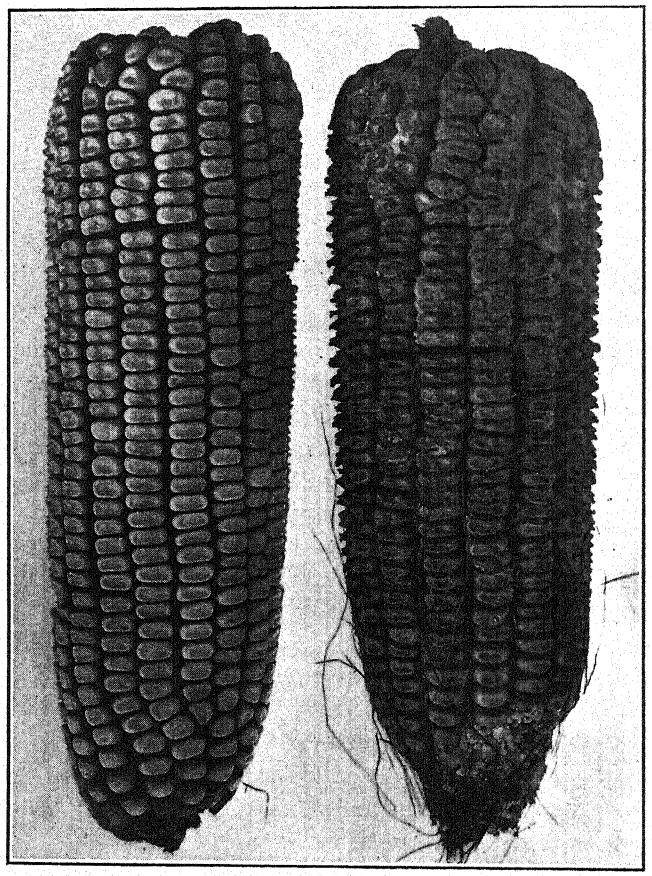


FIG. 1. Ear rotted by *Diplodia macrospora* (right) and a normal ear (left).

Leaves and stalks: Diplodia macrospora attacks the leaf sheaths and occasionally the leaf blades. Infected areas on these parts appear water-soaked to yellowish at first and later become brown, when the tissue is killed by the fungus. The disease may appear on any part of the blade and sheath, although the latter is most often infected at the base. The

lesions on the blade are irregular elongate in shape and may coalesce and form dead spots two inches in length and a half inch in width. The spots usually bear a few scattered pycnidia of the organism (Fig. 2, A).

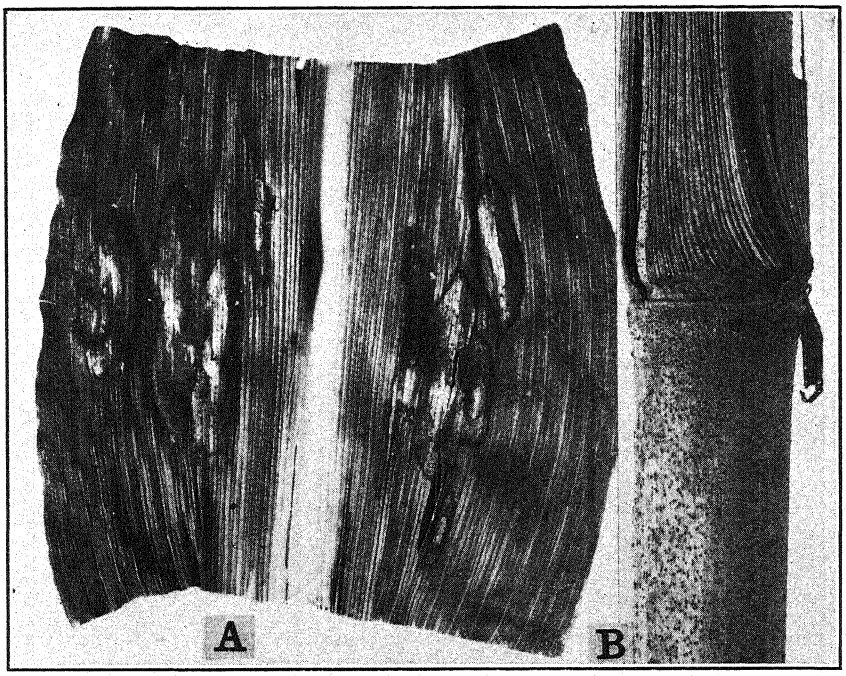


FIG. 2. A. Corn leaf showing pycnidia of *Diplodia macrospora* on diseased areas. B. Old corn stalk showing pycnidia of *D. macrospora* on the internode and node.

The fungus may attack the stalk at any node or internode, but the lower part of the plant is most often involved. The occurrence of pycnidia of the organism on the surface of the stalk at nodes and internodes is shown in figure 2, B. Stalks that are severely rotted at the lower nodes are sometimes blown down by the wind.

Roots: The disease has not been observed on the roots of plants in the field. However, the roots of seedlings grown in inoculated soil in pots in the greenhouse were rotted by the fungus. The invaded roots turned brown, shrank, and died, and the plants were either stunted in growth or killed.

CAUSAL ORGANISM

Morphology: The pycnidia of *D. macrospora* occur scatteringly on the dead parts of the corn plant (4) and are embedded in stromatic masses of mycelium and in host tissue, becoming erumpent at maturity. They are carbonaceous and flask-shape and measure 150–450 μ.

Immature pycnospores are granular and, when mature, are light brown, and frequently contain oil drops. They are borne on short hyaline conidiophores which arise from the inner cell wall tissue of the pycnidium and, at maturity, are discharged, under favorable conditions, through the elongated canal or ostiolum. The spores are cylindrical to club-shape and slightly curved to straight and consist of one, two, three, or four cells (Fig. 3, D), the two-cell type being the most common. They measure 43–95 x 6–13 μ, mostly 60–75 x 8–10 μ and average 67.8 x 9.0 μ.

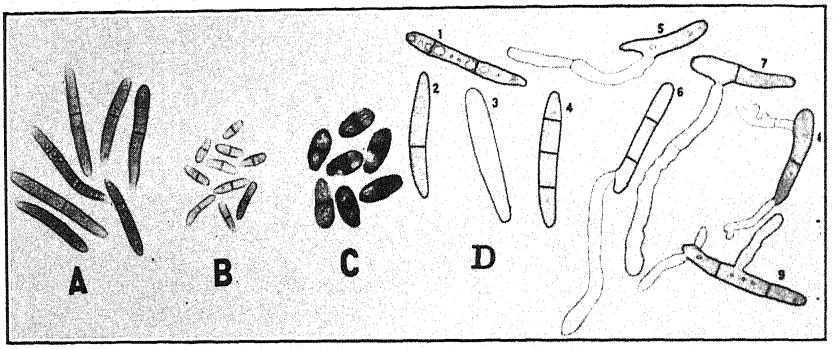


FIG. 3. A, B, C. Photomicrographs of spores. ×200. A. *Diplodia macrospora*. B. *D. zeae*. C. *D. frumenti*. D. Camera-lucida drawings of spores of *D. macrospora*: 1–4, various types of spores; 5–9, various types of spores germinating.

The spores germinate in water within 16 hours at 28° C. to 32° C., and each one of the cells of a spore may form a germ tube. The germ tubes and hyphae are at first hyaline and later become granular, septate, and branched. The growth of colonies of the organism on potato-dextrose agar and malt-extract agar is rapid, since only four days are required for a colony to become 9 cm. in diameter. The mycelium is dense and white.

Taxonomy: Diplodia macrospora and *D. zeae* (3) produce the same type of disease symptoms on the plant. Kernels of ears visibly infected with *D. frumenti* are blackened and often covered with a dark brown to black mold, while kernels infected with *D. zeae* and *D. macrospora* are seldom blackened but have a lusterless appearance and are often dull gray. Pycnidia of *D. frumenti* appear in clumps on the stalk and break out through longitudinal cracks in the epidermis (6), while those of the other two species usually occur singly and rupture the epidermis only at the point of their emergence.

The three species can be distinguished from each other on the basis of certain morphological characters, which are given in table 1 and shown in figure 3, A, B, C. *Diplodia frumenti* differs from the other two species in

TABLE 1.—*Comparison of some of the morphological characters of D. macrospora, D. zeae, and D. frumenti*

Species	Color of mycelium	Shape of pycnospores	Number of cells in pycnospores	Color of mature pycnospores	Dimensions in microns of 200 pycnospores of each species	
					Limits in dimensions	Average Dimensions
D. macrospora ...	White	Cylindrical to club; slightly curved to straight	1 to 4	Light brown	43–95 x 6–13	67.8 x 9.0
D. zeae	White	Cylindrical to club, rarely elliptical; slightly curved to straight	1 to 3	Light brown	17–33 x 3– 7	23.8 x 5.3
D. frumenti	Brown	Elliptical	1 to 2	Dark brown	19–31 x 11–15	25.3 x 13.3

color of mycelium and in the shape, color, septation, and dimensions of the spores. The mycelium of *D. macrospora* is white, like that of *D. zeae*, and the spores of the two species have the same color and shape. The only outstanding difference between the two species is that the spores of *D. macrospora* are from two to three times as long and about twice as wide as those of *D. zeae*.

Growth on culture media: Diplodia macrospora grew well at room temperature on corn-meal, carrot, oatmeal, lactose, potato-dextrose, and malt-extract agars in petri plates inoculated with uniform bits of mycelium from pure culture. There was little mycelial growth on glycerine agar. At the end of thirty days, pycnidia were formed on all of the above media except lactose agar. The organism also grew well on sterilized corn, wheat, and oat kernels and formed pycnidia in abundance within 68 days from the date of inoculation.

Relation of temperature to spore germination: Spores were germinated in water in drop cultures exposed to temperatures ranging from 5° C. to 39° C. in electrically controlled chambers in which the temperatures did not vary more than plus or minus one degree. Observations made at the end of 24 hours showed that the optimum-temperature range for germination was 25° C. to 32° C., as is shown in figure 4, A. Spores exposed for 6 days at 8° C. germinated 24 per cent, while no germination occurred at 5° C. during the same period, thus showing that the minimum temperature for germination lies between 5° C. and 8° C. The maximum was not ascertained.

Relation of temperature to mycelial growth: A series of plates of potato-dextrose agar were inoculated in the center with uniform bits of fungus mycelium and were incubated in triplicate for 4 days at different temperatures ranging from 5° C. to 39° C. The diameter of the colonies was measured at the end of 2 and 4 days and the average diameter of the three plates at each temperature is shown graphically in figure 4, B. The optimum temperature for growth of *D. macrospora* lies between 25° C. and 32° C. The maximum lies between 35° C. and 39° C., and the minimum between 5° C. and 8° C. It is interesting to note that the cardinal temperatures for the growth of *D. macrospora* closely approached those of *D. zeae*, as determined by Durrell (3).

Relation of hydrogen-ion concentration to mycelial growth: A 3 per cent potato-dextrose agar was used as the medium to determine the effect of hydrogen-ion concentration upon the rate of growth of the fungus. Thirty test-tubes, each containing 15 cc. of the medium, were sterilized and adjusted to different hydrogen-ion concentrations ranging from pH 2.0 to 8.5, by adding the required number of drops of 2N, N/1, and N/3

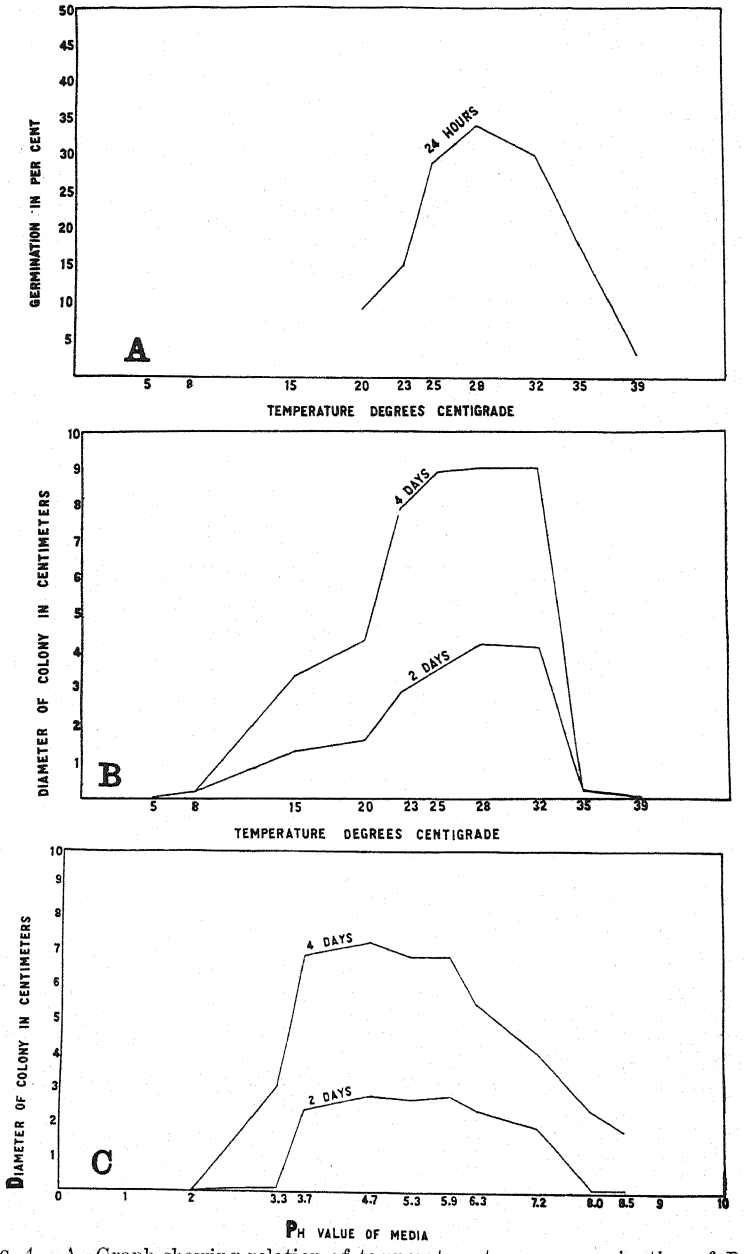


FIG. 4. A. Graph showing relation of temperature to spore germination of *Diplodia macrospora*. B. Graphs showing relation of temperature to mycelial growth of *D. macrospora*. C. Graphs showing relation of pH reaction of media to mycelial growth of *D. macrospora*.

HCl and N/1 NaOH. The amount of acid and alkali required for the different concentrations was determined with the Youden Hydrogen-Ion Concentration Apparatus. Petri plates were prepared in triplicate for each reaction, inoculated in the center with uniform bits of fungus mycelium, and exposed at a constant temperature of 30° C. for 4 days. The average diameter of the colonies of each reaction at the end of 2 and 4 days is shown graphically in figure 4, C.

The results show that the pH reaction most favorable for growth lies between pH 3.7 and 5.9. No growth occurred at pH 2.0 and the organism grew slowly at pH 8.5.

Pathogenicity. Ears: Diplodia macrospora proved to be parasitic on ears of corn artificially inoculated when in the soft-dough stage. In the season of 1928, 44 ears were inoculated by inserting a kernel of corn on which a pure culture of the fungus was growing into a wound made in the husks and pressing it down in contact with the kernels (7). Forty-three of the 44 ears were severely rotted by *D. macrospora,* which formed pycnidia on the outer husks. Forty-eight ears inoculated by the same method in 1929 also were severely rotted by the fungus.

In another test, ears were inoculated by spraying a water suspension of spores on the exposed tips of ears and by hypodermically injecting this inoculum into the husks near the butt end (1, 7). Four of the 19 ears inoculated at the tip were infected and rotted by the fungus, and 7 of the 19 ears into which the spores were injected were also severely rotted. These results indicate that, under natural conditions, *D. macrospora* may enter the ear through exposed tips and through wounds. In nature, ears are most commonly infected at the butt end, and wounds at this point are not essential for infection to occur, for the fungus can grow into the ear from primary infections in the ear shank and in the husks near the butt end. Thus, ears may become infected by *D. macrospora* in the same manner as they are infected by *D. zeae,* as described by Durrell (3).

Seedling roots: Healthy corn seedlings with roots an inch to two inches long were set out in sterilized white lake sand and in sand sterilized and inoculated with a pure culture of *D. macrospora.* At the end of two weeks the roots of the seedlings in inoculated sand were severely rotted by the fungus. The infected roots were shrunken and light brown and the tops of the plants were either wilted or dead. Plants grown in noninoculated sand had healthy roots and a normal top growth. In this experiment the amount of inoculum was greatly in excess of what normally occurs in the field, and the test indicates the parasitic capabilities of the fungus and not its actual pathogenicity for seedling roots in the field (2, 3).

Overwintering: Diplodia macrospora survives the winter in Florida in old plant débris. Spores collected from old stalks left in the field until May

germinated readily and produced ear infection. The organism also lives over as dormant mycelium in the kernels. Its longevity beyond one year has not been determined, but, like *D. zeae* (1, 3), it is probable that *D. macrospora* is capable of living several years on old plant refuse and in the soil.

SUMMARY

Diplodia macrospora causes a dry rot of the ears, husks, ear shanks, stalks, leaves, and seedling roots of corn in Florida. This disease can be distinguished from dry rot caused by *D. frumenti* on the basis of symptoms, but the symptoms of dry rot caused by *D. zeae* are similar to those caused by *D. macrospora,* and the diseases can be distinguished only by comparing the size of the spores of the two organisms from diseased parts of the corn plant.

The chief morphological difference between *D. macrospora* and *D. zeae* lies in the size of the spores. *Diplodia frumenti* differs from these two species in color of mycelium and in color, shape, size, and septation of the spores.

Diplodia macrospora grows well and forms pycnidia on standard culture media.

The optimum temperature range for spore germination and mycelial growth of the organism is 25° C. to 32° C., and the minimum lies between 5° C. and 8° C. Spores germinate at 39° C., but there is no mycelial growth at this temperature.

Diplodia macrospora grows best on potato-dextrose agar at pH 3.7 to 5.9. The minimum pH reaction lies between 2.0 and 3.3 and the maximum lies above pH 8.5.

The organism enters the ears through exposed tips and wounds and by growing through the shanks. It hibernates as dormant mycelium in the seed and in old plant débris and in the soil.

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PHYTOPATHOLOGICAL NOTES

A new method of sterilizing wood blocks to be used for the culture of wood-inhabiting fungi. The surfaces of wood blocks are readily sterilized by exposure to the fumes of glacial acetic acid. Following is the recommended procedure: Cut the wood into blocks of the size desired for cultures and place them in a glass dish containing an open vessel filled with glacial acetic acid; cover the dish; and leave the wood exposed to the acid fumes for six hours. At the end of three hours turn the blocks in order that all points on the surface of the wood may be reached by the acid. At the end of six hours the surface of the wood will be sterilized.

A culture dish 10 inches in diameter, provided with a cover and fitted with stands on which to rest the wood blocks, makes a suitable container for the sterilization of 4 blocks, 1 inch x 1 inch x 3 inches in size. Satisfactory stands to hold the blocks may be made by bending the ends of glass rods at right angles to the rods and fitting them into corks, which will act as supports to maintain the rods in a horizontal position in the sterilization vessel. An open dish containing the acid may be placed below such stands.

In the course of sterilization the surface of the wood will have absorbed some of the acid fumes; and, before the blocks are removed to the vessel in which they are to be inoculated, it is necessary to free them of the disinfectant. The fumes diffuse readily into the air. Transfer the blocks, therefore, to a dry, sterilized vessel into the air of which the acid may pass; if the presence of acid is still discernible after a few hours, transfer the blocks to a second sterilized vessel. When all the perceptible acid has disappeared, pass the blocks through several changes of sterilized distilled water to insure the removal of every trace of acid. This will, in addition, condition the wood to a moisture content suitable for fungous growth. It is well to pass the blocks somewhat rapidly through the first changes of water, leaving them not more than an hour in the first, in order that no trace of the acid may soak into the interior of the wood.

The method of sterilization here outlined is superior to sterilization by heat, since subjection of wood to heat for a period of time sufficient to insure the destruction of fungus spores or mycelium adhering to the surface changes to some extent the chemical composition of the wood and, therefore, alters the food supply of any fungus with which it may be inoculated. It must be remembered, however, that the acid sterilizes the surface of the wood only and that, therefore, thoroughly sound wood must be selected at the outset. It is well to run control cultures containing treated but noninoculated blocks, in order to ascertain whether or not any active fungus is present in the wood before inoculation.

All the fungi that I have attempted to grow on culture blocks prepared as here described have developed well. These are three in number: *Trametes pini* Fr. inoculated into blocks of jack pine, *Pinus Banksiana* Lamb.; *Torula ligniperda* (Willk.) Sacc. on blocks of paper birch, *Betula alba,* var. *papyrifera* (Marsh.) Spach.; and *Fomes igniarius* Fr. also on paper birch, *Betula alba,* var. *papyrifera.*

Trametes pini reduced sound jack pine to an advanced white-pocket stage of red rot and produced sporulating fruit surfaces on the blocks in a period of eight months. *Torula ligniperda* has caused a distinct discoloration of the birch blocks to a depth of 3/16 inch in the course of three months and has doubtless penetrated much farther into the wood. This fungus is sporulating freely in the interior of the blocks. *Fomes igniarius* is spreading rapidly over the surface of the wood, but the inoculations are recent, and none of the blocks have yet been removed from the culture flasks.—CLARA W. FRITZ, Timber Pathologist, Forest Products Laboratories of Canada, Ottawa, Canada.

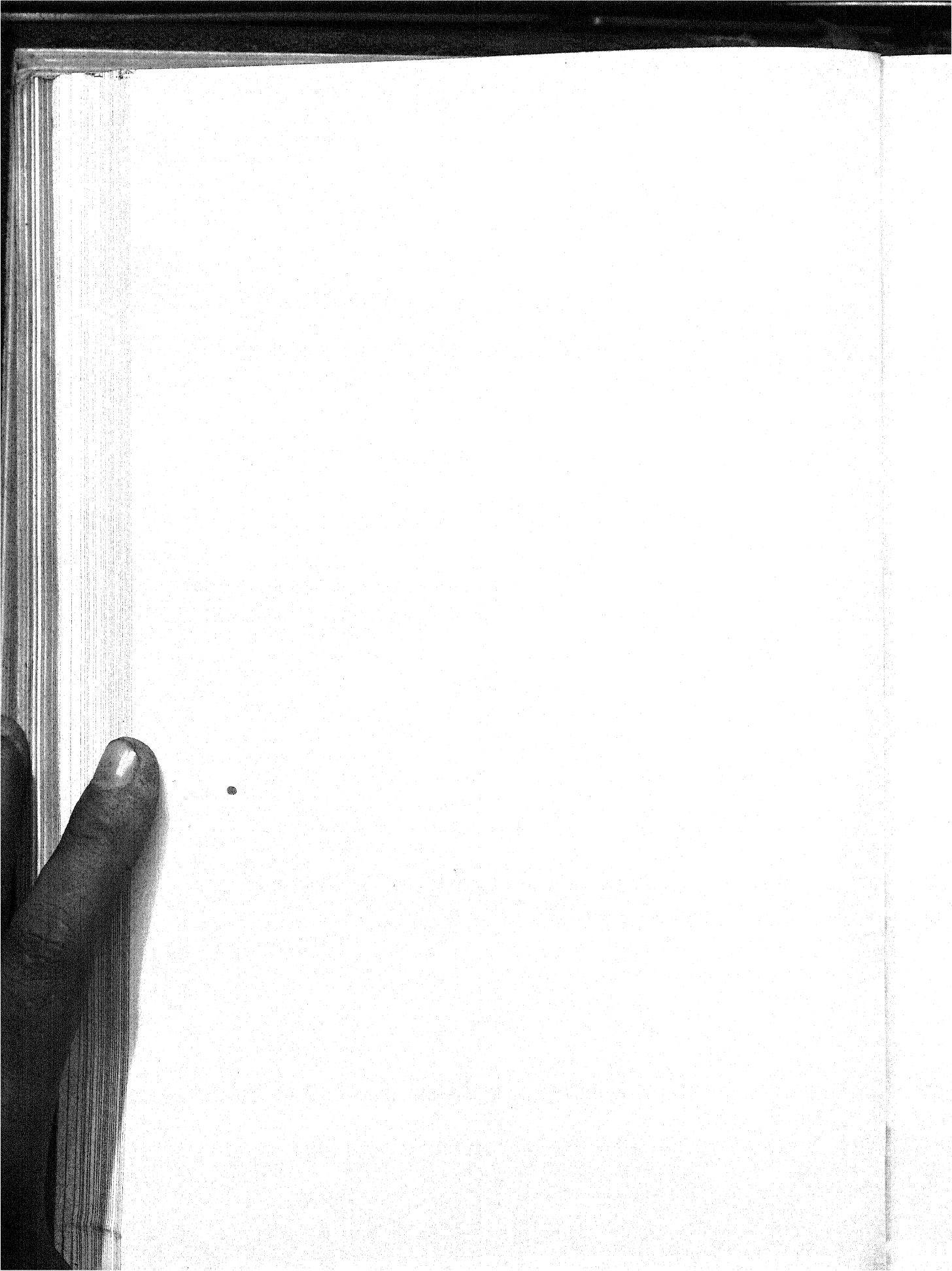
Additional data on the distribution of two species of Sphaceloma. It now seems certain that the distribution of the Sphaceloma on avocado, the cause of avocado scab, includes the country of Mexico. This statement is based upon the writer's identification of the fungus on avocado fruits recently recovered by Inspectors of the United States Department of Agriculture Plant Quarantine and Control Administration from the baggage of passengers at El Paso, Texas, entering the United States from Mexico. The organism had formerly been reported from Peru and Cuba, as well as from Florida. Its possible occurrence in Mexico, where the avocado is indigenous, would seem almost unquestionable but an earlier record has not been found.

Reports of a Sphaceloma on apple and pear in Europe have been received only from Russia and Switzerland. Its identification on apple fruits taken during the past six months by Inspectors of the Plant Quarantine Administration from the baggage of passengers coming to this country from both Italy and Ireland shows that this organism occurs also in these two countries; that is, presuming that the apples in each instance were grown in the country whence they were transported. The finding of a Sphaceloma on apple, apparently from Ireland, together with other available data to be reported later, suggests that this organism may cause the British "spot-disease" of the apple, the cause of which has not been definitely determined. The fungus is not known to occur in the United States. —ANNA E. JENKINS, Bureau of Plant Industry, Washington, D. C.

Notice of Summer Meeting. The Southern Division of the American Phytopathological Society will hold its annual Summer Meeting June 11–16, 1930. The arrangements for the field tour are under the direction of Dr. L. E. Miles, Chairman, and L. M. Fenner, Secretary. The group will assemble at the Walthall Hotel in Jackson, Mississippi, during the evening of June 11. On June 12, an early departure will be made southward to the truck-crop areas around Crystal Springs and Hazlehurst. This is the center of the fresh-tomato district. Field operations, grading, and packing may be observed. Tomato shipments will be at their height. In the field, observations will be made on the overwintering of bacterial canker of tomato and other tomato diseases. Diseases of beans, carrots, cotton, and sweet potatoes will be noted here and in the coastal area. Search will be made for the newly discovered phony disease of the peach. Departing from the famous health resort at Browns Wells on June 13, visits to pecan groves and nurseries will be made and the work of the South Mississippi Experiment Station at Poplarville will be noted. Citrus, figs, grapes, peaches, sugar cane, and gladiolus will be of interest here. From Hattiesburg, on June 14, the tour will include diseases of citrus, pecans, and sugar cane, arriving in Biloxi late that afternoon. Visits to truck-crop areas, nurseries, and the pecan station will be made on June 16.

For members interested in the collecting of fungi, this tour should afford an opportunity for gathering unusual specimens. The winter and spring seasons have been cool and rainy over much of the State and the conditions may favor a considerable number of plant diseases.

The State Plant Board of Mississippi and the Agricultural Experiment Station have tendered their services in making the tour available to visitors who may come by train. All members, visiting scientists, and agricultural workers interested in plant diseases and their control are extended a cordial invitation to meet with us. Requests for reservations should be addressed to the Committee at A. & M. College, Mississippi.



REPORT OF THE TWENTY-FIRST ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE DES MOINES MEETING

The American Phytopathological Society held its twenty-first annual meeting from Saturday through Tuesday with an attendance of about 150. Saturday and Tuesday meetings were held at the Grant Club, with headquarters at the Chamberlain Hotel. Monday sessions were conducted at Iowa State College, at Ames.

The program consisted of ninety-three papers delivered before the Society's sections and five delivered at two joint sessions, one with Section G of the American Association and the other with the Society of American Bacteriologists. The contributions may be classified as follows: cereal diseases, twenty-five; vegetable diseases, thirty-two; fruit diseases, thirteen; miscellaneous diseases, twelve; diseases of ornamentals, nine. There were two invitation papers, one by Christine Buisman, on the Dutch elm disease, and the other by R. P. White, on the future of the pathology of ornamentals.

The annual banquet on Saturday evening, 163 persons present, will long be remembered because of the clever program of original songs, impersonations, etc., rendered by members from Iowa, Minnesota, and Wisconsin.

On Sunday evening was held the second annual meeting with business men and farmers, the discussion this year being devoted to the timely subject: "What Present Trends in Agriculture Demand of Plant Pathology." Thomas Roberts (General Milling Company) spoke on the great west and wheat; the nation's greatest crop, corn, was discussed by H. A. Wallace (Wallace Publishing Company); the sugar-beet industry was represented by W. H. Baird (American Beet Sugar Company); and a paper on conserving the intensive crops prepared by C. G. Woodbury (National Canners Association) was read.

The Monday sessions were held at Iowa State College, at Ames, where there was an elaborate exhibit of experimental work. Displays of virus diseases, crown-gall investigations, and seed-corn treatments were noteworthy. There were three simultaneous sessions Monday morning and a joint session in the afternoon with the Society of American Bacteriologists, before which papers were given on bacteriophage and plant and animal viruses.

A sum of money equivalent to that taken in for the Endowment Fund Luncheon, which was a feature of the day, was contributed to the fund by the Iowa State College group. Following the luncheon, members present as guests contributed generously.

The extension pathologists held a round-table discussion on Tuesday with R. E. Vaughan, of Wisconsin, as chairman. The importance of conducting disease-control projects in close cooperation with other extension work was emphasized.

OFFICERS AND REPRESENTATIVES

The following officers were chosen:

President, H. S. Fawcett, Citrus Experiment Station, Riverside, Calif.

Vice-President, Max W. Gardner, Indiana Agricultural Experiment Station, La Fayette, Ind.

Councilors (two years), H. P. Barss, Oregon Agricultural Experiment Station, Corvallis, Ore.; W. D. Valleau, Agricultural Experiment Station, Lexington, Ky. (1 year to complete term of Max W. Gardner.)

Associate Editors (three years), Ruth F. Allen, University of California, Berkeley, Calif.; Mary K. Bryan, U. S. Department of Agriculture, Washington, D. C.; L. R. Hesler, University of Tennessee, Knoxville, Tenn.; Wm. H. Weston, Jr., Harvard University, Cambridge, Mass.

Business Manager (one year), F. C. Meier, U. S. Bureau of Plant Industry, Washington, D. C.

Advertising Manager (one year), James F. Adams, Agricultural Experiment Station, Newark, Del.

Representatives on the Council of the American Association for the Advancement of Science (one year), D. Reddick, Cornell University, Ithaca, N. Y.; C. W. Edgerton, Louisiana Agricultural Experiment Station, Baton Rouge, La.

Members of the Board of Governors of the Crop Protection Institute (three years), L. M. Massey to replace H. W. Anderson.

The following temporary committees were appointed to serve throughout the meetings:

Auditing Committee, R. E. Vaughan and J. B. Kendrick.

Committee on Elections, L. M. Fenner and Reiner Bonde.

Committee on Resolutions, H. W. Anderson, R. P. White, and J. G. Leach.

De Bary Memorial Program Committee, Mel T. Cook, F. M. Blodgett, L. E. Melchers.

REPORT OF THE SECRETARY-TREASURER, 1929

The Society started the year 1929 with 794 members. During the year 4 past members have been reinstated and 29 have been lost. Of the 29, 15 have been suspended for nonpayment of dues, 8 have resigned, and 4 have died, 2 of those elected at the New York meeting not having paid their dues. This makes a net loss of 25 and a total membership of 769 at the close of 1929.

There are now 84 life members paid in full—54 paying currently and 631 regular annual members. I have at this meeting applications for 59 and if these are elected it will bring our total membership up to 828.

The State of Minnesota leads in securing new members for 1929 with six applications, Iowa, New York, and Wisconsin tying with five each; Indiana, Washington, and Canada, three each; Illinois, District of Columbia, Hawaii, and Michigan, each two; and the remainder scattered through nine States, and the following foreign countries: England, Greece, India, and Japan.

Our estimate of a working balance of $899.74 for 1930 is ample to take care of the probable needs of the Society.

STATEMENT OF ACCOUNTS FOR 1929, AS OF DECEMBER 15, 1929

Receipts:

Balance from 1928		$2,361.61
Annual dues: 1927	$ 4.00	
1928	24.25	
1929	1,628.20	
1930	2,125.40	3,781.85
Excess dues		.50
Interest on checking account		45.42
Sales received with dues		4.11
Donation to Lyman Memorial Fund received with dues		13.00
Total receipts		$6,206.49

Expenditures: (As per vouchers attached)

Member subscriptions transferred to PHYTOPATHOLOGY (1929)	$2,178.00	
" " " " " (1930)	500.00	
Secretarial work	288.38	
Postage stamps and stamped envelopes	68.45	
Stamped printed envelopes	68.22	
Expenses of Secretary-Treasurer at New York meeting	66.01	
Printing (abstracts, preliminary announcements, ballots, nomination ballots, final programs, tickets, letterheads)	208.71	
Incidental expenses (account books, buttons)	11.70	
Donations to Lyman Memorial Fund to Savings Account	25.00	
Supplies for and mimeographing Publicity abstracts	32.10	
Sales transferred to PHYTOPATHOLOGY	10.44	
Checks returned by bank	46.00	
Bank charges for collection of checks	1.20	
Balance as shown by bank statement attached	2,702.28	
		$6,206.49

SUPPLEMENTARY STATEMENT

Balance as above stated			$2,702.28
Amount of above receipts due PHYTOPATHOLOGY:			
For member subscriptions, 1928	$ 3.00		
1929	63.00		
1930	951.00		
For sales received with dues	.54	$1,017.54	
Amount due Sinking Fund:			
For 1925, 1927, 1928	306.00		
For 1929	522.00		
For 1930	378.00		
		1,206.00	
			2,223.54
Available working balance for 1930 expenses			$ 478.74
Balance from 1929 as per attached statement		$ 478.74	
Estimated income from annual regular members for 1930		372.00	
Estimated interest on December checking account		4.00	
Estimated interest on 1930 checking account		45.00	
			$ 899.74

During my first year as Secretary-Treasurer I have received invaluable assistance from our President, Dr. R. J. Haskell, who, in 1928, at the close of six years' active service, requested relief from this office. It was also my good fortune to have the assistance of Miss Mary G. Van Meter. Her knowledge of the work and her keen interest in the welfare of the Society, combined with a willingness to devote practically all of her spare time to PHYTOPATHOLOGY, contributed much to our year's program.

Respectfully submitted,

F. C. MEIER, *Secretary-Treasurer.*

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1929

STATEMENT OF ACCOUNTS FOR 1929, AS OF DECEMBER 15, 1929

Receipts:

Balance from 1928		$ 636.54
Subscriptions ($795.51 for 1930)		3,079.34
Sales, PHYTOPATHOLOGY	$ 803.09	
Phytopathological Classics	7.37	
		810.46
Advertising: 1927	23.00	
1928	47.15	
1929	880.63	
		950.78
Interest on Sinking Fund		352.50
Member subscriptions for 1929		2,178.00
Member subscriptions for 1930 (in part)		500.00
Donation to Lyman Endowment Fund		25.00
Donation from National Academy of Sciences		500.00
State of West Virginia, share in cost of Leonian article		425.50
Tropical Plant Research Foundation for colored plate		445.95
Member dues received with subscriptions		5.00
		$9,909.07

Expenditures:

Manufacturing PHYTOPATHOLOGY:			
Vol. XVIII, No. 11	$ 434.24		
Vol. XVIII, No. 12	470.15		
Vol. XVIII, Index	184.43		
		$1,088.82	
Vol. XIX, No. 1	654.06		
Vol. XIX, No. 2	438.75		
Vol. XIX, No. 3	787.52		
Vol. XIX, No. 4	962.39		
Vol. XIX, No. 5	719.03		
Vol. XIX, No. 6	485.08		
Vol. XIX, No. 7	459.63		
Vol. XIX, No. 8	384.78		
Vol. XIX, No. 9	1,027.37		
Vol. XIX, No. 10	633.34		
Vol. XIX, No. 11	430.25		
Vol. XIX, Cuts	913.45		
		7,896.55	
			8,985.37
Expenses of Editor in Chief			113.50
Postage of Editor in Chief			102.48
Secretarial work			89.25
Insurance on stock of back volumes at Lancaster, Pa.			18.00
Postage in mailing back volumes			14.84
Expenses of Advertising Manager			33.24
Donation Lyman Fund transferred to Savings Account			50.00
Duplicate subscriptions refunded			15.25
Balance as shown by bank statement attached			487.14
			$9,909.07

Last year our cash balance in the PHYTOPATHOLOGY account was $636.54, this year it is $487.14. We have paid for eleven numbers of the 1929 Journal but in doing so we have expended 1930 receipts from subscriptions to the amount of $795.50 and we have transferred $500 of the member subscriptions for 1930 from the Society account to help meet this cost. We still have the December number and the Index to pay for and they will probably cost $800, also bills for the commission allowed the Advertising Manager and for stenographic work to the amount of $133.80, making approximately $933.80 due for 1929 expenses and this amount must be paid from 1930 receipts.

Assistance in the publication of PHYTOPATHOLOGY was received this year in the form of a $500.00 grant from the National Academy of Sciences Fund for Publication of the Results of Research.

SINKING FUND

The Sinking Fund composed of six dollars for each sustaining life-membership is now $6,456.00 of which $6,250.00 is invested in first-mortgage notes. The remaining $206 is in the Society's checking account and will be invested as soon as the life-membership dues for 1930 have been received.

ENDOWMENT FUND

The endowment fund established and named for Doctor George Richard Lyman now totals nearly $1,500[1] in cash donations and pledges. Of this amount $828.44 is placed in a savings account with the McLachlen Banking Corporation. There have been cash contributions ranging from $100 to $1.00. Pledges have been received in amounts from $10.00 per annum for 10 years to $1.00 per annum for 5 years.

STATUS OF SUBSCRIBERS

Our edition of PHYTOPATHOLOGY is 1,625 of which 1,267 copies are mailed to members and subscribers. The number of subscribers in good standing at the close of the year is 498, of which 172 are domestic and 326 are foreign. A glance at the record of subscribers for the past ten years shows a growth of 222.

Respectfully submitted,

F. C. MEIER, *Business Manager.*

REPORT OF THE EDITOR IN CHIEF

During the current year the editorial staff of PHYTOPATHOLOGY has conscientiously striven to maintain the high standard of excellence to which our journal has been brought through the labor and devotion of others who have served as its editors. Much credit is due Dr. E. C. Stakman and his associate editors for assistance rendered the editor in chief and his staff in editing the manuscripts and attending to editorial responsibilities attendant upon the publication of the January, February, and March numbers. Grateful acknowledgment also is made on behalf of the helpful criticism and editorial and other valuable assistance rendered by different members of the present editorial staff and by J. Marion Shull, Frances W. Todd, and others.

The 19th volume of PHYTOPATHOLOGY comprises 1,147 pages of printed matter and illustrations classified as follows: Eighty-eight articles, 19 phytopathological notes, 2 reports, 2 book reviews, 38 plates, and 199 text figures. From January 1, 1929, to December 20, 1929, approximately 130 manuscripts were submitted. Of this number, 31 were returned to the authors for revision, 6 were rejected, and 3 were withdrawn by their authors. Volume 19 contains a total of 125 phytopathological abstracts and 15

[1] Two hundred nineteen dollars was received at the Endowment Fund Luncheon at Ames, Iowa, on December 29, 1929. This sum does not appear in the above statement.

abstracts by title only. Of the several manuscripts received during the year 1929, 22 articles, 4 phytopathological notes, 2 book reviews, and 98 abstracts are now in press.

During the past year an effort has been made and, thanks to the helpful cooperation of The Science Press Printing Company, some success has been realized in advancing the date of delivery of our journal to its subscribers. Before another year passes, it is hoped each month's issue will be mailed on the first of the month.

Certain of our membership have expressed the thought that we should formulate and adopt a more definite editorial policy with reference to character and length of articles accepted for publication in PHYTOPATHOLOGY, publication of notes, announcements, book reviews, interim reports, quarantine regulations, etc. Accordingly, a questionnaire was prepared and sent out to a limited number of members, more especially to those who have served or are serving the Society in an editorial capacity. These questionnaires have been returned and from them an attempt will be made to formulate a policy with reference to all points covered in the list of questions.

It is the aim of the editorial staff to cooperate to the utmost in the improvement of the scientific excellence and wide usefulness of our journal. Therefore, no pains will be spared in our effort to obtain manuscripts of exceptional quality both as to subject matter and excellence of exposition. Greater care should be exercised by our contributors in the selection of adequate illustrations of such excellence as to guarantee the best possible reproduction. Contributors should also devote more attention to the clarity of presentation of tabular data and to terseness in statement of scientific facts. Much editorial labor has been expended during the past year and in past years in an effort to publish within the limits of our budget. This has necessitated not a little pruning out of extraneous matter and exclusion of tabular material that could be and has been more advantageously incorporated in the text, proper. Again, we would call attention to the fact that authors of papers can profit through reference to recent numbers of PHYTOPATHOLOGY from which they may learn what is required in the preparation of tables, graphs, and illustrations. Attention to such details and to a more careful preparation of the text will go a long way toward insuring prompt publication of manuscripts.

H. B. HUMPHREY, *Editor.*

REPORT OF THE ADVERTISING MANAGER

In the twelve issues of PHYTOPATHOLOGY published during the year 1929, there appeared a total of 113 advertisements distributed as follows: 38 one-page, 52 one-half-page, 20 one-fourth-page, and 3 one-eighth-page advertisements. The total advertising was equivalent to 69⅜ full pages, or an increase over last year of six and two-thirds pages.

Soliciting advertising is a continuous responsibility each month of the year in order to maintain our small total of business. Renewal of contracts secured for 1930 at this time totals only 36 pages, which further indicates the necessity for continued soliciting and the need of cooperation on the part of members to assist in this work if we expect to maintain a reasonable advertising business.

It is suggested that members write the Advertising Manager of any favorable prospects and whenever in touch with commercial representatives to mention the opportunity PHYTOPATHOLOGY offers as a practical medium for advertising their equipment and materials directly to the members with whom they wish to do business.

J. F. ADAMS, *Advertising Manager.*

COMMITTEE ON INVESTIGATION OF FOREIGN PESTS AND PLANT DISEASES

Your Committee has devoted its time during the past year as follows:

1. To the preparation of a list of important plant diseases based upon an early list prepared by J. A. Stevenson.

2. To conferences between members of the Committee and Secretary A. M. Hyde, Dr. A. F. Woods, Drs. W. A. Taylor and K. F. Kellerman, and other influential leaders in Washington.

3. To correspondence between chairmen of the two coordinate committees whereby it was agreed to present a joint resolution together with substantiating data to Secretary Hyde requesting that an appropriation for this purpose be included in the budget for 1931.

4. To redrafting the resolution adopted by this Society at its last session so that it forms a joint resolution from the Association of Economic Entomologists and the American Phytopathological Society.

5. To the presentation of this joint resolution, together with supporting data, through letter of transmittal to Secretary Hyde signed by members of both committees.

6. To following up the progress of the resolution. At the last report the matter was being considered by the Budget Committee.

C. R. ORTON, *Chairman*,
W. A. MCCUBBIN,
F. D. FROMME.

COMMITTEE ON QUARANTINE AND REGULATORY WORK

This Committee, consisting of W. A. McCubbin, Chairman, J. S. Boyce, J. F. Adams, H. T. Güssow, and M. W. Gardner, submitted the following report, read by Doctor Gardner.

At the present time there appears to be no great demand or need for regulating importations of pathogenic cultures from foreign countries.

The unsatisfactory condition of quarantine aims and policies is noted.

Unification of policies, standards, and practices in nursery inspection seems desirable.

The following resolution is proposed:

Whereas: The American Phytopathological Society cordially endorses the policy of the United States Department of Agriculture in reference to competent and impartial investigation of all outbreaks of foreign plant diseases and insect pests in this country in order that effective and reasonable control measures may be adopted, be it

Resolved: That this Society desires to express its approval of the action taken by Secretary Hyde, of the Department of Agriculture, in appointing advisory groups of well-qualified scientists from various States and institutions to survey and report on the Mediterranean fruit-fly situation in Florida both as to the need and the adequacy of the control measures adopted, including research, eradication, and quarantine.

W. A. MCCUBBIN, *Chairman*.

COMMITTEE ON COOPERATION WITH BIOLOGICAL ABSTRACTS

Your Committee on Biological Abstracts consisting of J. I. Lauritzen, Chairman, L. O. Kunkel, G. W. Keitt, S. M. Zeller, and H. T. Güssow, held no meeting during the year and so can give you only a brief summary of the progress made by Biological Abstracts as reported by Dr. J. R. Schramm.[1]

During the year some 36,000 abstracts were published and 45,000 were sent to press. Beginning with January, 1930, regular monthly publication will be established.

[1] Read by Dr. L. O. Kunkel.

Each number will contain about 3,000 abstracts. It is hoped that the index for Volume 1 can be published sometime next spring.

One of the most important achievements of the past year was the establishment of a branch office in the Library of the U. S. Department of Agriculture. This library has made available $5,000 a year for salaries and $5,000 a year for serials desired both by Biological Abstracts and the U. S. Department of Agriculture. The number of current serials to which the staff of Biological Abstracts has access has been brought to about 5,500. Twenty-five thousand are available at the Academy of Natural Sciences in Philadelphia, 1,200 are at the College of Physicians in Philadelphia, 1,200 are at the central office of Biological Abstracts, and 600 are in the Library of the Department of Agriculture. There are at present over 3,000 collaborators. Dr. Schramm pleads for our continued support. The Committee heartily recommended that all possible support be given to Biological Abstracts during the coming years.

J. I. LAURITZEN, *Chairman.*

COMMITTEE ON EDITING PHYTOPATHOLOGICAL ABSTRACTS

On the basis of the acceptance by the Society of the report made by the Committee on Editing Phytopathological Abstracts December 31, 1928, and published in PHYTOPATHOLOGY, Vol. 19, pp. 520–521, 1929, the Committee has this year applied the following rules:

1. November 15 is the closing date for receipt of manuscripts for phytopathological abstracts for the annual meeting and this is interpreted strictly to mean that the abstracts must be in the hands of the Committee on or before that date.

2. Late receipt of manuscripts and length in excess of 200 words will act automatically to disqualify.

3. Manuscripts presenting material which, in substance, has already been published will not be accepted.

4. The Society has previously ruled that not more than two abstracts under the sole or senior authorship of one person shall be accepted.

It has been necessary this year to reject certain manuscripts because of failure to observe one or another of these rules.

The vexing question of the proper disposition of progress reports, which are in effect only a compilation of a greater or smaller portion of the routine observations, spraying tests, and miscellaneous experimentation performed during the year, still remains. This Committee recognizes that sometimes more can be made of such papers by their actual presentation than is apparent from the preliminary abstract and that they sometimes evoke discussions mutually beneficial to author and audience. We recognize that the presentation of papers of this character is sometimes employed as a means to place one's name on the program and that their acceptance may be an important factor in determining attendance at the meetings. It is not the place or purpose of this Committee to attempt to legislate with respect to the papers which appear on the program, but we hold that a considerable proportion of this sort of material is not scientific literature in a proper sense and should not be published as if it were. Although the Committee is authorized to reject manuscripts which contain no new contribution to knowledge, experience shows that it is very difficult to secure general agreement among the Committee that any manuscript is so deficient. The practice has been to let nothing less than the unanimous judgment of the Committee determine the rejection of a manuscript. As a result we continue to publish abstracts in which no substantially new result, process, or thought is discernible, and the present year is no exception. This situation is discreditable to the high scientific aims of the Society.

We therefore propose the adoption of another rule supplementary to the four rules above stated, as follows:

5. The Committee may at its discretion order printed by title only such manuscripts as do not of themselves give evidence of a new contribution to scientific knowledge.

These five rules will govern the Committee's action with respect to the manuscripts submitted in 1930 and thereafter. It is proposed to publish these rules in a number of PHYTOPATHOLOGY issued prior to October 15 each year, and a synopsis of them in the preliminary notice of the meetings.

FREEMAN WEISS, *Chairman,*
J. W. ROBERTS,
ANNIE R. GRAVATT,
CHARLES DRECHSLER.

COMMITTEE ON PHYTOPATHOLOGICAL CLASSICS

With respect to Phytopathological Classics I can only report that due to the financial stringency in the treasury of PHYTOPATHOLOGY, I have made no attempt the past year to present manuscripts for publication in this series. I can, however, report some progress. Doctor Humphrey tells me that he has the manuscript of the translation of Tillet's paper on cereal smuts on which he has been working for some time practically completed. This can be gotten ready, I take it, for publication within a short time after I am advised that there is money available for printing it. I shall be glad to be advised by the Council as to the prospects of having funds for publishing new numbers of the Classics. As soon as money is again available, this series should be pushed with vigor.

H. H. WHETZEL, *Chairman.*

COMMITTEE ON PRESS

During the year stories were released to the *West Virginia Agriculturalist* and to the alumni magazines of Dartmouth College and Harvard University on the late Dr. G. R. Lyman and the George Richard Lyman Endowment Fund for Phytopathological Publications. The report of the Des Moines meetings, published in *Science,* was released by the Press Service.

The most important phase of the work of the year, and that for which the Press Committee was established, was the preparation of paper-by-paper releases of the Des Moines meetings. Each paper on the program, in so far as the Secretary received abstracts, was rewritten in popular language, and, following the final arrangement of the program, session stories were prepared, each with its proper release date, and mimeographed. Copies of the several stories were mailed to over two hundred newspapers, press associations, farm journals, and feature writers.

Following the meetings at Des Moines, a story of the new officers of the Society, together with photographs in so far as they were available, was released to the Associated Press.

W. A. WHITNEY, *Chairman.*

COMMITTEE ON INTERNATIONAL RELATIONS

Your Committee, appointed to inquire into the desirability of seeking to develop an international organization for the furtherance of our science and the promotion of international cooperation and good will, reports as follows:

1. It has been determined by consultation with a representative gathering of our membership that the time seems opportune for the formation of an international organization.

2. It is recommended that the President appoint a committee (the number to be left to his discretion) to begin at once preliminary negotiations looking to a conference of interested persons, the conference to be held at Cambridge, England, at the time of the International Botanical Congress, in August, 1930.

3. A plan of organization, approved in principle by an informal conference of the Society in session at Des Moines on December 29, 1929, is presented for the information of the proposed new committee.

D. REDDICK, *Chairman*,
H. S. JACKSON,
J. C. WALKER,
H. S. FAWCETT,
G. H. COONS.

COMMITTEE ON NECROLOGY

The decease of four members is reported: Franklin Sumner Earle, January 31, 1929; Charles W. de Rekowski, April 16, 1929; Dr. Leigh H. Pennington, April 24, 1929, and Dr. W. H. Wright, May 3, 1929.

On account of their achievement and personal qualities, these members had won the professional respect and personal friendship of their colleagues. The American Phytopathological Society records its sense of loss in the death of these members. The formal report will be found at the end of this record.

A. G. JOHNSON, *Chairman*,
G. P. CLINTON,
M. B. WAITE.

REPORTS OF OTHER COMMITTEES AND REPRESENTATIVES

Committee on American Type Culture Collection. I submit herein a brief report for the year 1929 of the fungus section of the American Type Culture Collection maintained in the Office of Mycology and Disease Survey, Bureau of Plant Industry.

No. of transfers sent to Dr. Weaver during 1929	199
No. of cultures in collection as report of Dec. 23, 1928	457
No. of cultures in collection Dec. 31, 1929:	
Aspergillus	46
Penicillium	36
Phycomycetes	66
Pythium and Phytophthora	34
Miscellaneous	319
Total available for distribution	501

There are also several dozen recently acquired cultures not yet placed in the collection for distribution.

In June, 1929, a supplementary list of 113 cultures of fungi was sent to Dr. Weaver's office. Since that supplement was compiled about 25 cultures have been placed in the collection.

During the year a culture cabinet large enough to hold 1,200 cultures has been constructed to facilitate handling of the cultures. A minimum of trouble from loss or contamination was experienced during the year.

C. L. SHEAR, Representing Phytopathological Society.

American Association for the Advancement of Science. A brief, informal report of the representatives on the *Council of the American Association for the Advancement of Science* was presented by D. Reddick.

Auditing Committee. We, the undersigned Auditing Committee, have examined the books of the American Phytopathological Society, American Journal of Phytopathology, and the Lyman Endowment Fund. The books are found in good order with receipts and expenditures as stated by the Secretary-Treasurer, Mr. F. C. Meier.

R. E. VAUGHAN and J. B. KENDRICK.

De Bary Memorial Program Committee. A committee consisting of Mel T. Cook, F. M. Blodgett, and L. E. Melchers brought in the following report, which was adopted by the Society:

Your Committee on the de Bary Memorial Program for the next meeting recommends:

1. That the American Phytopathological Society invite the Mycological Section of the Botanical Society of America to join in a one-session program to be known as the de Bary Memorial Program.

2. That the secretaries of these two organizations be authorized to arrange said program.

3. That so far as possible the papers for said program be on subjects along the lines of de Bary's work. These papers may be selected from the papers submitted or by invitation, as may appear most satisfactory to the secretaries.

MEL T. COOK, *Chairman*,
F. M. BLODGETT,
L. E. MELCHERS.

Resolutions Committee. A committee consisting of H. W. Anderson, R. P. White, and J. G. Leach brought in the following resolutions, all of which were adopted.

Resolved: That the American Phytopathological Society, assembled at Des Moines, Iowa, instruct its Secretary to extend to the newly formed Canadian Phytopathological Society, on behalf of its members, its congratulations upon the organization of the Society and its heartiest felicitations for continued growth and prosperity.

Resolved: That the members of the American Phytopathological Society hereby extend their sincere thanks to Dr. I. E. Melhus and other members of the Local Arrangements Committee for their efficient efforts contributing to the success of the 1929 convention of the Society held at Des Moines, and Ames, Iowa.

Resolved: That the American Phytopathological Society express its appreciation to the Des Moines Chamber of Commerce, through its Secretary, Mr. George Hamilton, for its generous help and cooperation during the preparation for and the holding of its 1929 annual meeting.

Resolved: That the Society express its appreciation to the Grant Club of Des Moines for the hospitality extended to the Society at its annual 1929 convention.

Resolved: That the Society express its appreciation of the facilities and assistance rendered by the public schools and Boy Scout organization of Des Moines during its annual convention.

Whereas: It has been called to our attention that Prof. W. J. Morse, of the Maine Agricultural Experiment Station, long an active and devoted member

of our Society, is at present seriously ill, *Be It Resolved,* that the Secretary be instructed to extend to him the sympathy of the Society and its hope for his early recovery.

ACTION OF THE COUNCIL

In addition to making the appointments mentioned in the first part of this report, the Council made the following recommendations, which were approved by the Society.

1. That inasmuch as a change in the Constitution would be necessitated, no special class of membership for commercial companies be recommended at this time.

2. That the invitaton of H. W. Anderson and M. W. Gardner for a summer field meeting in southern Illinois and Indiana be accepted and that a committee consisting of M. W. Gardner, H. W. Anderson, and Leslie Pierce be appointed to take charge of arrangements. (Owing to extensive damage from winter injury to fruit trees in these States, this meeting has since been canceled.)

3. That the Editor of PHYTOPATHOLOGY be allowed actual expenses for 1930 up to or within $300 for secretarial and editorial assistance. This is the same amount as last year.

4. That a committee be appointed to consider plans for the Chicago Exhibition of 1933, and, if thought desirable to enter such an exhibit, to bring in a plan at the beginning of the next annual meeting.

OTHER BUSINESS

The reports of officers, committees, and representatives, as printed above, were accepted.

The revised copy of the Constitution and By-Laws was ordered printed in PHYTOPATHOLOGY. The matter of printing a revised list of members was referred to the Council with power to act.

The Secretary read letters from T. G. Major notifying the members of the dissolution of the Canadian Division of the American Phytopathological Society and the organization of the Canadian Phytopathological Society. It was moved and carried that the Resolutions Committee draft a reply to the Canadian organization expressing our best wishes to the new organization.

B. M. Duggar announced that "Fifty Years of Phytopathology" (illustrated) by Erwin F. Smith, an invitation address before the International Congress of Plant Sciences, Ithaca, New York, August 20, 1926, is now available and may be obtained at 75 cents per copy by addressing him, University of Wisconsin, Madison, Wis.

Attention was called to the fact that the publication "Fungi of Iowa," by Gilman and Archer, may be purchased from the Business Manager of the Iowa State Journal of Science at $1.00 per copy.

A. H. R. Buller announced that "Selecta Fungorum Carpologia," by Tulasne, has been translated from Latin to English by W. B. Grove, the author of the "British Rust Fungi," and that the translation, along with reproductions of the plates, is now being published by the Oxford Press.

E. C. Stakman, speaking in behalf of the Endowment Fund Committee, brought up the matter of the desirability of the phytopathologists availing themselves of the opportunity to obtain group rates and special service from steamship companies for travel to the Botanical Congress and he pointed out that the Endowment Fund might well benefit from this arrangement, which means the equivalent of one free ticket for every twenty-five purchased. Following a discussion of this matter, G. H. Coons made a motion, which was carried, that some member of the Society be appointed as corre-

sponding agent of the U. S. Lines in order that the Endowment Fund might benefit and the individual members secure the special service offered to groups buying tickets. It was moved by E. L. Nixon and carried that G. K. K. Link,[1] of the University of Chicago be made sole correspondent in this matter.

It was moved by Donald Reddick and carried that, with the probability of an International Union being effected at the Botanical Congress in Cambridge, the matter of delegates be referred to the Council for action at the next meeting with a view to determining a permanent plan for financing delegates to the International Union if one be founded.

It was moved by Donald Reddick and carried that, in view of the fact that our new President is now to appoint committees, and with them one to go to Cambridge, and is abroad and therefore inaccessible, Dr. Haskell, retiring President, be made Chairman of the Committee on International Relations and that he select the other members of that committee. (The committee was reappointed with R. J. Haskell as Chairman.)

[1] Doctor Link is now actively carrying on this work, is in close touch with the steamship company, and inquiries should be addressed to him.

FRANKLIN SUMNER EARLE

SEPTEMBER 4, 1856—JANUARY 31, 1929

Franklin Sumner Earle took special courses at the University of Illinois and in 1902 received the degree of M.S. from the Alabama Polytechnic Institute.

In 1886 he was an assistant to Doctor Burrill in the Department of Botany of the University of Illinois; from 1894 to 1895 he was connected with the Mississippi Agricultural Experiment Station. In 1895–96 he was Assistant Pathologist in charge of the Mycological Herbarium of the U. S. Department of Agriculture. In 1896 he was Horticulturist at the Alabama Agricultural Experiment Station; in 1896–1901, Professor of Biology at the Alabama Polytechnic Institute; 1902–3, Assistant Curator in charge, Mycological Collections, New York Botanical Garden; 1904–6 Director, Estacion Central Agronomica of Cuba; 1908–11, Consulting Agriculturist to the Cuban-American Sugar Company; 1911–14, President, Cuba Fruit Exchange; 1918, studied sugar-cane mosaic in Porto Rico for the U. S. Department of Agriculture; 1922–23, Consulting Agriculturist to the Central Aguirre Sugar Company, Porto Rico. From 1924 until his death he had charge of the study of sugar-cane varieties for the Tropical Plant Research Foundation in Cuba.

He was a man of sterling character and friendly disposition, a devoted husband and father, greatly respected by his business and professional associates and acquaintances, and beloved by his more intimate colleagues and friends.

CHARLES W. DE REKOWSKI

SEPTEMBER 8, 1865—APRIL 16, 1929

Charles W. de Rekowski received his education in the Gymnasium and the University of Jena, Germany, where he specialized in agriculture. In 1866 he came to the United States where he became interested in the sugar-beet industry and was active and well known in that industry throughout the balance of his life. He was a member of a number of scientific societies and social clubs in different parts of the United States and was highly thought of by his many friends.

LEIGH H. PENNINGTON

OCTOBER 26, 1877—APRIL 24, 1929

Leigh H. Pennington received the degree of Bachelor of Arts from the University of Michigan in 1907 and the degree of Doctor of Philosophy from the same institution in 1909, specializing in botany and chemistry.

During the school year 1906–1907, he served as undergraduate assistant in botany at the University of Michigan and continued in the same position during his graduate study at the same institution, from 1907 to 1909. From 1909 to 1910, he was Instructor in Botany at Northwestern University. From 1910 to 1912, he was Assistant Professor at New York State College of Forestry. From 1912 to 1914, he was Associate Professor at the same institution and from 1914 to the time of his death, he was Professor of Forest Pathology in the same institution. During the summers of 1911 and 1912, he served as expert in the Office of Forest Pathology, U. S. Department of Agriculture, and at various intervals during 1917–1923, he served as pathologist or collaborator in the same office. During the summers of 1923, 1925, 1926, 1927, 1928 and at the time of his death, he served in the capacity of forest pathologist in the Office of Blister-Rust Control, U. S. Department of Agriculture, working on special phases of the white-pine blister-rust problem.

He was the author of numerous papers, chiefly in the field of forest pathology. He was a very able, far-seeing investigator, capable teacher, and willing co-operator, combining, in all, thoroughness and a very pleasing personality. Truly it may be said of him that "To Be of Service" was his watchword.

WILLIAM HARMON WRIGHT

JULY 8, 1885—MAY 3, 1929

William Harmon Wright graduated from Purdue University in 1908 and married Edna Fisher June 11 of the same year. He came to the University of Wisconsin that fall and received his Master's degree in 1909. His graduate work at Wisconsin was supplemented by a year at Cornell University, 1915–1916. The degree of Ph.D. was awarded him by the University of Wisconsin in 1924.

Professor Wright joined the staff of the University of Wisconsin as an assistant in Agricultural Bacteriology. He was successively promoted through the various ranks, occupying at the time of his death the position of Associate Professor of Agricultural Bacteriology.

He was an exceptional teacher. A thorough mastery of his subject and a delightful personality, activated by a glowing enthusiasm, enabled him to inspire, not only a large number of students, who came under his direction during his twenty years of service, but also those who were associated with him in his work. Although he had only a limited time for research, his zeal for original investigations and the fundamental work he accomplished were exceptional.

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STUDIES ON THE MOSAIC DISEASE OF THE BEAN (PHASEOLUS VULGARIS L.)

T. G. FAJARDO[1]

INTRODUCTION

Within the last decade the cultivation of both field and snap beans, *Phaseolus vulgaris* L., has increased markedly in certain sections of the United States. This has been associated especially with the increase of the bean-canning industry in the North and of the winter culture of beans in the South. At the same time there has been a concentration of the bean-seed-growing industry in the Northwest. Concomitant with these changes has come increasing loss from certain seed-borne diseases. Among these, the virus disease, "mosaic," ranks as most important on certain varieties. Although this has been recognized as serious for some time, but little progress has been made recently in its critical study, partly because of the difficulty in studying this mosaic experimentally.

The investigations here reported are the result of studies conducted at Madison, Wisconsin, dealing principally with certain phases of artificial and natural transmission of the disease, including transmission through the seed.

HISTORY, GEOGRAPHIC DISTRIBUTION, AND ECONOMIC IMPORTANCE

Bean mosaic was first reported in Russia by Iwanowski (10), in 1899, on a variety of *Phaseolus vulgaris*. Clinton (3), in 1908, described a questionable "infectious chlorosis" of string beans in Connecticut, which "in general appearance resembled very closely the chlorosis (mosaic or calico) troubles of tobacco, tomatoes and muskmelons." Spragg and Down (21) state that the disease was serious on the commercial navy bean in Michigan in 1908. In 1914 the disease was reported as occurring locally and causing slight damage in New York, but in 1916 and 1917 it became more general and severe (17). Barss (2) noted the disease in Oregon in 1917 and found that in certain fields as high as 50 per cent of the plants were affected.

[1] The writer is indebted to Professor L. R. Jones, at whose suggestion this investigation was undertaken, for advice and encouragement throughout the investigation; to Dr. James Johnson for helpful advice; and to Professor H. P. Barss, Dr. S. M. Zeller, and Dr. I. A. Hoggan for aid in the preparation of the manuscript.

Since the appearance of bean mosaic in Connecticut, Michigan, New York, and Oregon, the disease has been reported in various other States and is now apparently coextensive with bean culture in the United States (18) and perhaps in Canada (4, 8, 23). The disease has caused serious losses in the Eastern States in the past, but with the development of "Robust," a chance selection from Michigan, and other resistant varieties of dry shell beans, conditions have improved in this region. In the Western States, however, especially in Montana, Idaho, and other States where commercial seed beans are grown, but where Robust is not, the disease has become the most serious malady with certain important varieties. According to the Bureau of Plant Industry (Plant Disease Survey for 1925 to 1927), bean mosaic was ranked second only to bacterial blight in causing reduction in total yield for the bean crop of the United States (18).

While bean mosaic and the loss caused by it are coextensive with bean culture in America, only meager reports of the disease in other countries have been found in literature. Jørstad (12) reported its occurrence in Sweden in 1920, Ogilvie (16) in Bermuda in 1923, and Porter (19) in Eastern China in 1926. Quanjer, in a letter to the writer in 1927, states that he had observed it often in Holland and also in England. Since the disease is carried through the seed, it is probable that it occurs wherever beans are grown. Presumably the spread from one locality to another is chiefly through commercial shipments of bean seed.

SYMPTOMS

General symptoms and effects of bean mosaic. The symptoms displayed by bean plants affected with mosaic are variable and are influenced by various environmental conditions. In general, the leaves show varying degrees of mottling or chlorosis, blistering, and downward cupping of the lamina (Fig. 1, A, B, and E). Under certain environmental conditions, much-reduced leaves, with sinuous or filiform leaflets, are produced; in some cases only the terminal leaflet develops (Fig. 1, C, D). These latter symptoms, however, have not been observed in the field.

Plants affected with mosaic are rarely killed but continue to vegetate to the end of the season. They may become stunted, dwarfed, and bushy, or may be only slightly affected in growth, especially when the plant becomes infected at a later stage of development. In general, they shed their flowers more freely than do healthy plants and the setting of pods is delayed, the pods not all being set at the same time (Fig. 2). Under these conditions, the plants may be induced to more active budding and the vines appear "green" or take longer to "dry off" than the vines of healthy plants. This is especially noticeable towards the end of the season, when uniform

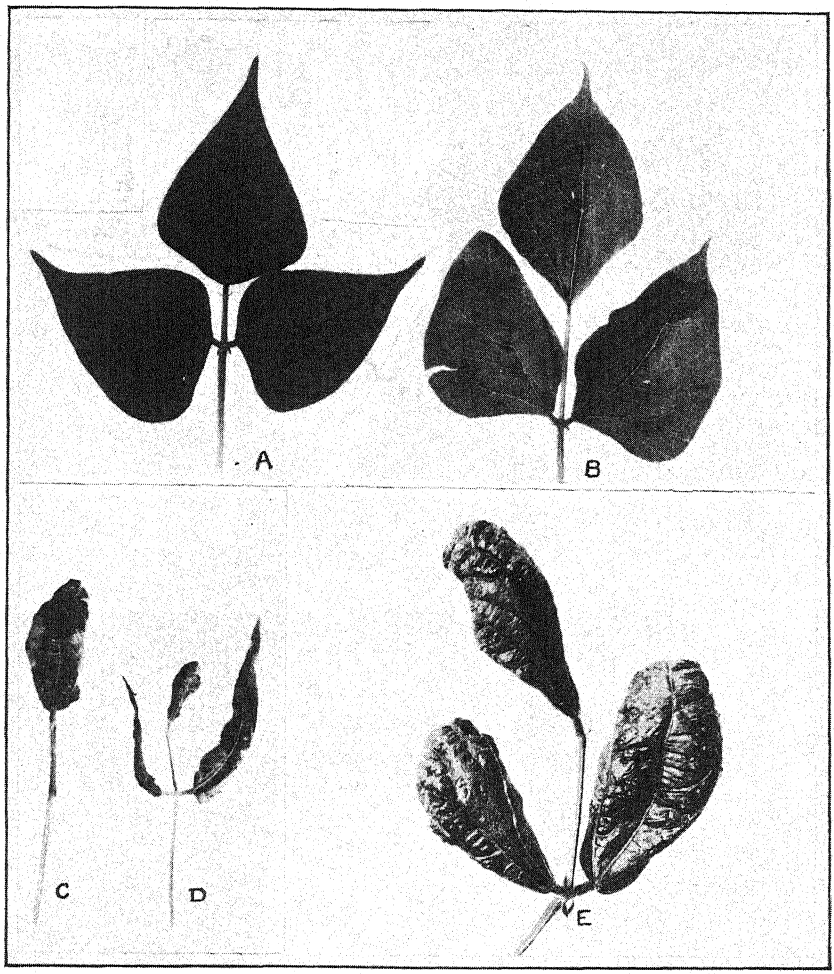


FIG. 1. Variation in leaf symptoms exhibited by different susceptible varieties of beans affected with mosaic.

Top row, Early Marrow Pea. A, leaf of healthy plant. B, leaf of mosaic plant showing chlorosis, with a few, small, dark green areas near the veins.

Bottom row, Rogers Stringless Green Pod. C, severe blistering and malformation of leaflet, with suppression of lateral leaflets. D, filiform malformation of leaflets. E, marked blistering and cupping of leaflets.

yellowing or drying of healthy vines occurs. Plants which become infected late in the season, however, may appear green although their pods mature uniformly and at approximately the same time as those of healthy plants.

The loss in yield, due to mosaic, varies from 0 to 100 per cent, depending on the stage of growth of the plant when infection occurs and on the relative

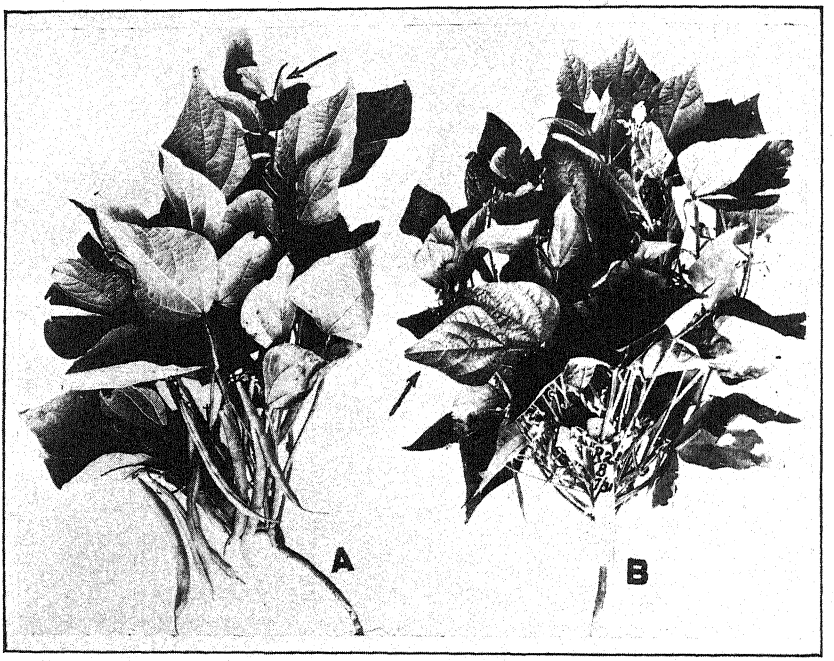


FIG. 2. The amount of injury caused by mosaic is much greater when infection occurs early. A, plant which developed primary symptoms (indicated by arrow) on August 20 and on which the yield was not reduced. B, plant which developed primary symptoms (indicated by arrow) on July 31, when in the fourth compound-leaf stage, showing delay in setting of pods and corresponding reduction in yield. (Refugee 1000–1 variety, photographed August 25.)

susceptibility and season of the variety. Plants infected early and developing severe leaf symptoms oftentimes fail to produce any pods. Plants infected at a later stage, before or just after blossoming, may be reduced in yield from 30 to 50 per cent, while plants infected after the pods are set are slightly or not at all reduced in yield (Figs. 2 and 3).

Symptoms on plants infected during the current season. Healthy plants which become infected during the current season through the agency of insects or through artificial inoculation show the first evident symptoms of mosaic on the young expanding leaves 8 to 15 days after inoculation. The youngest leaves which are expanding at the time of inoculation become crinkled, chlorotic, or stiff, with a much shortened petiole. The next youngest leaves are at first thickened, more or less chlorotic, and laterally arched, but later become completely chlorotic with petioles of normal length (Fig. 4, B). Under favorable greenhouse temperatures, these symptoms may be much more pronounced and a clearing of the veins may

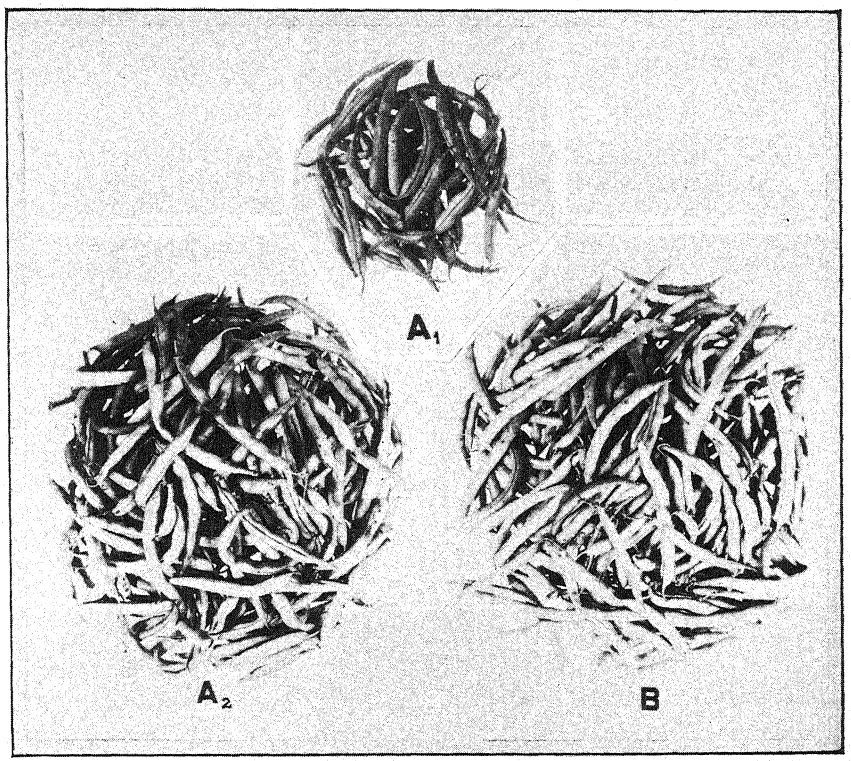


FIG. 3. Comparative yields of healthy and mosaic plants (Refugee 1000–1). A_1, yield from 5 plants infected at an early stage of growth. A_2, yield from 5 plants developing first symptoms of mosaic when in full blossom or later. B, yield from 5 healthy plants (control).

be observed (Fig. 5). Under certain conditions, however, plants infected during the current season may not develop typical symptoms. Such plants are either growing very slowly or have already reached full vegetative growth before they become infected, or they bcome infected late in the season when the weather is cool and moist. These latter plants may show only a crinkling or chlorosis of the last compound leaves; but, when conditions become more favorable for growth and the air temperature becomes higher, new leaves may be produced which show typical mosaic symptoms.

Symptoms on plants originating from infected seed. As will be discussed later, bean mosaic is often transmitted through the seed, although not all the seeds from an infected plant show mosaic infection. Seedlings originating from infected seed vary in the time of appearance and severity of leaf symptoms. Table 1 illustrates the results obtained with mosaic parent plants in relation to the time of appearance of symptoms in their

TABLE 1.—*Relation of time of infection of the parent plant to the percentage of seed infected. (Note the larger percentages result from early inoculation)*

Stage of growth of parent plant when infected	Number of experimental plants	Total number of seeds secured and tested	Percentage of seedlings from these developing mosaic symptoms	
			After 20 days[a]	After 30 days[a]
2nd to 4th compound leaf...	10	163	44	57
5th to 7th compound leaf.....	5	171	25	36

[a] Beginning from the date on which the seeds were planted.

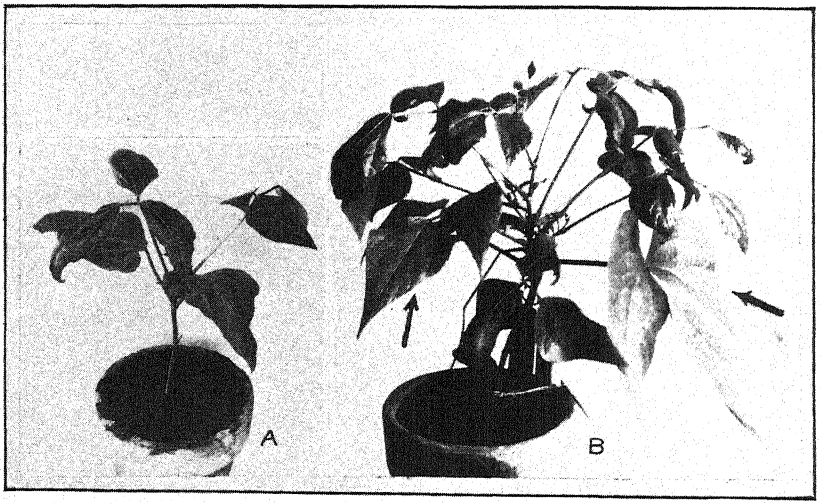


FIG. 4. A, seedling plant 20 days old, grown from infected seed and showing typical mosaic symptoms on both simple and compound leaves. B, plant artificially inoculated in the second compound-leaf stage, showing primary symptoms on the second and third compound leaves (indicated by arrows) and typical symptoms on subsequent leaves. (Roger's Stringless Green Pod.)

progeny. Our observations indicate that some seedlings develop typical mottling, blistering, downward cupping, or chlorosis on the first and succeeding compound leaves, while the two simple leaves are chlorotic, mottled or contorted, or else normal in appearance (Fig. 4, A). Sometimes only one of the simple leaves shows any symptoms. On other plants, however, even the first compound leaves do not develop typical symptoms but show a stiffening, crinkling, or chlorosis of the lamina, while the petiole becomes shortened and thickened and subtends the lamina in a more or less upright

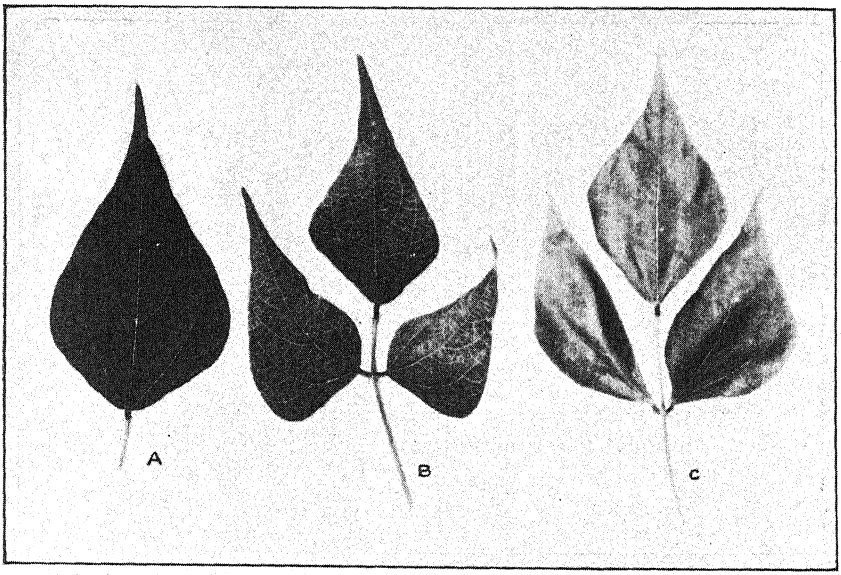


FIG. 5. Primary mosaic symptoms resulting from artificial inoculation in the greenhouse (Roger's Stringless Green Pod). A, leaflet of healthy plant. B, leaf of mosaic plant showing marked clearing of the veins. C, leaf of mosaic plant showing general chlorosis.

position. The second compound leaves are chlorotic, and typical symptoms develop on subsequent leaves. Thus far, it has not been possible to find any explanation of the fact that different plants of the same variety, grown from the same lot of infected seed and under identical conditions, should vary thus in the time of appearance of symptoms.

The severity of leaf symptoms on plants grown from infected seed was found to vary widely according to the variety. Generally speaking, the early, apparently resistant, varieties do not show such well-marked mottling, blistering, or cupping as is observed on the late susceptible varieties. The early, apparently resistant varieties, such as Bountiful, Davis White Wax, Pencil Pod Wax, Wardwell's Wax, and Giant Stringless Green Pod, show only a slight mottling or chlorosis of the leaves. Sometimes the symptoms on the compound leaves are obscured in the greenhouse, even though the simple leaves show marked contortions. Under greenhouse conditions these varieties blossom very early, which may explain the failure in some cases of the appearance of mosaic symptoms. Our experience indicates also that both these varieties and the late-maturing types, unless inoculated early, may fail to develop mosaic symptoms.

Influence of air temperature on the expression of mosaic symptoms. Air temperature affects the general symptoms of bean mosaic in much the

same way as it affects those of other mosaic diseases (11). Bean seedlings with marked mosaic symptoms were placed in greenhouses and chambers held at different temperatures. Good symptoms developed on the new growth at 20–28° C., a masking of symptoms at 12–18° C., and a partial masking at 28–32° C. At 20–28° C., the plants apparently grew normally, at 12–18° C. they were stocky, and, at 28–32° C., they grew more rapidly and were spindly. Field observations during 1926 and 1927 confirm these results. Marked symptoms were noted in the field from about the last half of June to the first two weeks of August, when the mean temperatures ranged between 20 and 29° C. Before and after this period, when the mean temperatures lay between 10 and 18° C., the symptoms were indistinct or were completely masked. The masking of symptoms was more noticeable late in the summer when cooler weather ensued.

The incubation period is also affected by the air temperature. Under good growing conditions, inoculated plants kept at 20–28° C. showed primary symptoms within 8 to 14 days. Typical symptoms appeared 3 to 6 days later. Plants kept at 28–32° C. showed symptoms in approximately the same range of time. Inoculated plants kept at 12–18° C. showed a lengthening of 4 to 7 days in the incubation period and failure to develop typical symptoms even after 25 days. When transferred to a temperature of 22–26° C., however, typical symptoms appeared in 10 days. In these experiments from 60 to 100 per cent infection was obtained with plants kept at 12–18° C. and at 20–28° C. Much lower percentages of infection were obtained with plants kept at 28–32° C. The low percentages of infection obtained with inoculated plants at the higher temperatures may have been due to the sensitiveness of the virus to drying or to the rapid drying of the mutilated leaves at these temperatures.

Judging from the above results, the favorable temperatures for the expression of mosaic symptoms are 20–28° C., with an optimum between 22 and 26° C.; partial masking occurs at 28–32° C., or above, and total masking at 12–18° C. At favorable temperatures (20–28° C.) typical mosaic symptoms develop within 15 to 20 days, a much shorter period than that given by Stewart and Reddick (22), which was 4 weeks.

IS THERE MORE THAN ONE KIND OF BEAN MOSAIC?

Since great variations in mosaic symptoms have been found among the different varieties of beans and since field-infected plants under certain conditions fail to develop typical mosaic symptoms during the season, one might be led to suspect the existence of more than one kind of mosaic on bean. The writer, however, is at present of the opinion that there is only one common type of mosaic of the bean. It has not been possible to make

extensive intervarietal cross inoculations and studies of the properties of the virus derived from different sources. This conclusion, however, is based, first, on the results of a study of symptom development in a series of experiments with young susceptible plants, where inoculations were made with infective materials taken from plants and varieties showing diverse symptoms in the field, and, second, on observations of the diverse effects of the disease on different plants raised from diseased seeds taken from the same infected parent plant. The variations observed in the expression of symptoms seem to be due to differences in host reaction as influenced by a variety of internal and external factors.

DISTRIBUTION OF THE VIRUS IN INFECTED PLANTS

The virus of bean mosaic is perhaps present in the whole system of mosaic-infected plants, but its presence is most easily demonstrable in those plant parts which exhibit mosaic symptoms. Artificial inoculations with juice from the leaves, stems, or green pods of mosaic-infected plants readily produce infection in young, healthy bean plants. No infection has thus far been obtained from the juice of roots of infected plants. Several attempts to inoculate young seedlings with water extracts from dried seed coats of mosaic-infected seed carrying 60 to 75 per cent seed infection gave negative results. Surface sterilization of infected bean seeds had no appreciable effect, and this held true whether or not the seed coats were removed before sterilization. This indicates that virus is, perhaps, not present in the seed coat but is carried in the embryo of the seed. No attempts have been made to inoculate with the juice from fresh seed coats. It is possible that the virus may be present in them, but, if so, it probably loses its virulence and becomes inactive with the drying of the seed coat.

TRANSMISSION OF BEAN MOSAIC

Transmission through the soil. In several series of experiments no transmission of mosaic was obtained through the soil. In these experiments seeds of susceptible varieties of beans were planted in soil mixed with fresh, dried, or decomposed mosaic tissues, or the seeds were allowed to sprout through a layer of fresh, mosaic-infected bean leaves. These results indicate that the virus of bean mosaic probably does not overwinter in dead, diseased vines and is perhaps never transmitted through the soil. As will be discussed later, the bean-mosaic virus remains viable an exceedingly short time and will not resist drying.

Transmission through contact with diseased and healthy roots. No transmission has yet been obtained in several series of experiments conducted in an attempt to infect healthy plants through contact with the

roots of mosaic plants. In these experiments seeds of healthy and mosaic plants were planted in the same pot. In no case could transmission be ascribed to root contacts. The healthy seedlings remained healthy, although the seeds from the mosaic plants gave a high percentage of infected seedlings.

Transmission through contact with the aerial parts of diseased and healthy plants. Leaves of healthy and mosaic plants, allowed to come in contact with each other and casually rubbed together under controlled conditions, failed to cause infection of the healthy plants. Alternate picking of green pods from mosaic and healthy plants grown under cages likewise gave no infection. The results of these trials indicate that bean mosaic is not readily transmissible through cultural practices, which must therefore be considered of little or no economic importance in the transmission of bean mosaic in the field.

TRANSMISSION BY ARTIFICIAL MEANS

Seed inoculation. No infection was obtained when dry or germinating seeds from healthy plants were soaked in juice expressed from diseased plants for different lengths of time. Pricking the cotyledons of germinating seeds before soaking in infective juice for 12 hours failed to induce infection. The juice was proved still to be infectious, after 12 hours, by inoculation to healthy plants.

Root inoculation. In two series of experiments, successful infection was, however, obtained when the cut roots of seedlings were immersed in infective expressed juice for various lengths of time. From 20 to 40 per cent infection was obtained on seedlings in the simple-leaf stage after such soaking for 1 to 1½ hours and on plants with the simple leaves half expanded after soaking 3 to 5 hours. No infection was secured on seedlings in the cotyledonary stage after soaking for 12 hours. Older plants subjected to this method died after replanting, as did some of the plants in the simple-leaf stage. These results suggest that infection resulted thus through cut roots only when sufficient transportation occurred through aerial parts to induce fairly active absorption and distribution of the virus.

Leaf inoculation. To date, bean mosaic compared with the well-known tobacco and cucumber mosaics, has been considered difficult to transmit artificially. Although transmission was secured by the earlier workers, the percentage of infection obtained was low and the results inconsistent (20, 7), while others failed to obtain transmission (6, 3). Clinton (3), failing to transmit the disease, regarded it as a "questionable chlorosis." Reddick and Stewart (20) state that they failed "in producing infection with expressed juice of diseased plants either with sufficient regularity or in

sufficient quantity to feel sure that the occasional plants showing the disease had not become infected in some other way.'' Archibald (1) states, however, that he obtained 100 per cent infection with hypodermic injections and by pressing the leaves between the fingers moistened with extracts from mosaic plants.

Using the methods of artificial inoculation known to transmit tobacco or cucumber mosaic and also repeating some of the methods used by the earlier workers with bean mosaic, the writer also has found the disease difficult to transmit. A method of leaf-mutilation inoculation has been developed, however, which has given from 80 to 100 per cent of infection in the majority of cases. This method consists in rubbing the simple leaves on the upper surface along the midrib with a small piece of cheesecloth dipped in infective, expressed juice to which a small quantity of sand has been added. Satisfactory results also were obtained by rubbing the leaves with mosaic tissues which were ground up and mixed with sand and rolled into small balls. These same methods applied to other small areas of the simple leaves have given satisfactory infection, but this has not been so commonly practiced as the rubbing of the midrib. The addition of sand in the juice, which is readily taken up with the cheesecloth, greatly aids uniform abrasion of the rather tough and firm leaves of the bean plant and avoids unnecessary tearing and crushing. By these methods, infection of susceptible varieties has been secured at any stage from the simple-leaf to the flowering stages.

Transmission by insects. The spread of certain virus diseases has been largely ascribed to insect agencies, and bean mosaic has been assumed by earlier workers to be transmitted by insects also. Our early field trials showed that if susceptible bean plants were so caged as to exclude insects they remained healthy through the season, while exposed controls developed mosaic symptoms before the end of the season. Later experiments, however, have proved more conclusively that certain aphids readily transmit bean mosaic, while all other various insects tested have failed to give infection.

Transmission by aphids. Hawley (9) states that the work of Matheson and Reddick indicated the transmission of bean mosaic by an undetermined species of plant louse in the greenhouse. Nelson (15) showed that *Macrosiphum solanifolii* Ashm., the pink and green potato aphid, transmitted bean mosaic. The results of a number of cage experiments with mosaic-reared *Aphis rumicis* L., the black bean aphid, *Myzus persicae* Sulzer, the peach aphid, and *Macrosiphum solanifolii*[2] have shown that each of these

[2] The writer wishes to thank Dr. A. A. Granovsky of the Department of Economic Entomology, University of Wisconsin, for kindly identifying the aphid species.

species readily transmits the bean-mosaic virus to healthy bean plants, confirming Nelson's results with the last-named aphid. Eighty to 100 per cent infection was secured when 15 or more infective aphids were transferred to each individual experimental plant. Lower percentages of infection were obtained when fewer aphids were used. In one series of experiments, infection was obtained with a single viruliferous individual of *A. rumicis* and like results with *Myzus persicae*. The incubation period remained within the usual range whether many or few aphids were transferred to each plant. Figure 6 is a general view of the plot where insect transmission experiments were conducted.

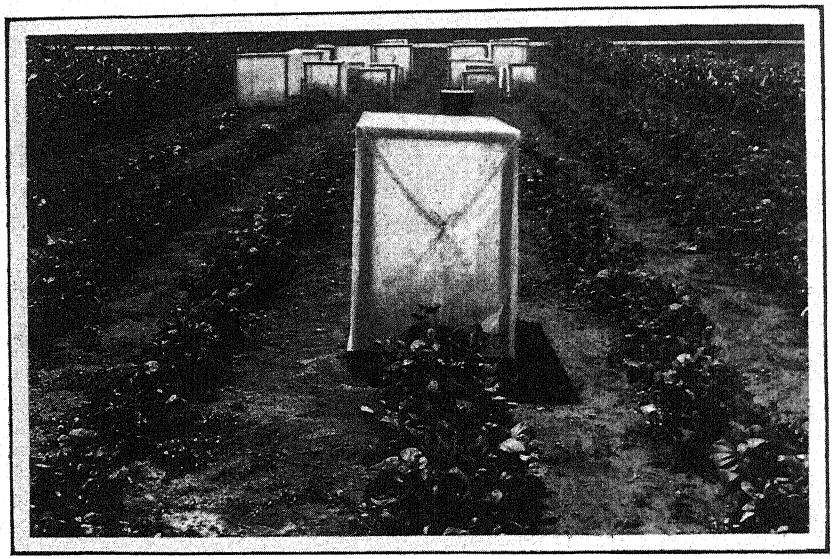


FIG. 6. General view of bean plot showing cages under which insect-transmission experiments were conducted.

Transmission by other insects. With one exception tests with various other insects found feeding on bean plants in the field gave negative results. Several trials with the bean leaf hopper, *Empoasca fabae* Le B., reared on mosaic bean plants, have given negative results. No infection was obtained either from *Diabrotica duodecimpunctata* Oliv., the twelve-spotted cucumber beetle, *D. vittata* Fabr., the striped cucumber beetle, *Lygus pratensis* L., tarnished plant bug, *Tetranychus telarius,* red spider, *Haliothrips fasciatus,* thrips, or *Aleyrodes vaporarium* Westw., white fly. Positive infection was obtained, however, with a species of mealybug in the greenhouse.

TABLE 2.—*Summary of results of 1926 trials as to the amount of field transmission attributed to insects in bean plots at Madison, Wisconsin. (Plantings made from June 3–5)*

Variety	Plot I			Plot II		
	Total No. of plants	Percentage of seed-borne mosaic	Percentage of mosaic plants on Sept. 15	Total No. of plants	Percentage of seed-borne mosaic	Percentage of mosaic plants on Sept. 15
Early Varieties						
Giant Stringless Green Pod	132	2.2	33.3	74	1.3	98.7
Bountiful	82	1.0	56.3	86	0.0	100.0
Davis White Wax	75	0.0	8.0	36	0.0	100.0
Improved Golden Wax	58	0.0	29.1	15	0.0	73.0
Refugee Wax	55	3.6	47.3	74	0.0	100.0
Dwarf Horticultural	105	4.7	35.0	64	0.0	100.0
Extra Early Refugee	108	0.0	38.9	67	0.0	100.0
Wardwell Wax	30	0.0	26.7	25	0.0	100.0
Full Measure	32	0.0	34.4	17	0.0	100.0
Grennel Wax	47	0.0	21.0	15	0.0	46.0
Late Varieties						
Refugee 1000–1	132	2.2	33.2	74	1.3	98.7
Roger's Stringless Green Pod	116	15.5	84.5	63	3.2	96.8
Hodson Wax	70	20.0	80.0	74	5.4	94.6
Early Marrow Pea	62	0.0	43.5	70	1.4	98.6
Large Marrow Pea	95	1.0	35.8	72	0.0	100.0
Robust	75	0.0	0.0	112	0.0	0.0

FACTORS INFLUENCING MOSAIC TRANSMISSION IN THE FIELD

The rate of spread and final percentage of mosaic infection in our experimental bean plots have been observed to vary from season to season. The determining factors appear to be (1) the percentage of mosaic-infected seed, and (2) the prevalence of certain insects. Climatic conditions may influence either the development of insects or the expression of mosaic.

In 1926 samples of seed of representative commercial varieties of beans, which carried low percentages of infection, were planted in early June in parallel rows in each of three different plots. The percentage of plants showing infection in two of these, plots I–II, at the end of the season, is given in table 2. The same variety showed differences in the percentage of infected plants in the different plots when final notes were made. The rows planted in plot II, where aphids appeared early in July and were more abundant during the season, gave a higher percentage of infection than those planted in the other two plots. In the latter plots aphids appeared late and were not abundant throughout the season. The rate of spread of mosaic was here much slower than in plot II. These results were confirmed by those obtained in 1927. In 1927 seeds from selected healthy plants were planted in rows alternately with mosaic seeds. Plot II showed 100 per cent infection by the end of July, while only a few cases of field infection were observed at that date in the other two plots. These other plots, however, also showed 100 per cent infection at the end of the season. The high percentages of field infection observed as compared with the previous year were due to the higher percentages of infected seed planted in the plots. The prevalence of aphids was similar to that of the preceding year.

INFLUENCE OF ROGUING ON AMOUNT OF FIELD INSPECTION

Roguing was found to have some influence on the prevalence of mosaic when the amount of seed infection was low and the roguing began with the earliest seedling symptoms before the appearance of aphids. Thus in one trial where, in the nonrogued controls, the infection of the more susceptible varieties reached 30 per cent, the corresponding rogued plots showed not more than 10 to 20 per cent even on the late susceptible varieties. Although this represented a reduction to almost one-half, it cannot be considered a practical measure under ordinary conditions.

Our experiments have shown that the amount of field infection attributed to insects and the resulting mosaic injury may vary widely according to date of planting. This is illustrated in table 3, which shows the results from each of three plantings, early June, late July, and early August. It will be noted that the two earlier plantings gave high infections on all

TABLE 3.—*Relation of date of planting to the amount of mosaic infection at the end of the season (1926 trials, Plot II). (Note the very small amount of mosaic in the August planting when there were few insects)*

Variety	Planted June 10		Planted July 23		Planted August 5	
	Total No. of plants	Percentage mosaic on Sept. 10	Total No. of plants	Percentage mosaic on Sept. 10	Total No. of plants	Percentage mosaic on Sept. 10
Early Varieties						
Refugee Wax	19	100.0	55	100.0	21	0.0
Giant Stringless Green Pod	24	100.0	20	100.0	15	13.3
Bountfiul	40	100.0	46	100.0	20	10.0
Davis White Wax	20	100.0	17	100.0	20	5.0
Pencil Pod Wax	30	66.7	22	75.0	25	8.0
Improved Golden Wax	15	73.3	25	100.0	15	0.0
Dwarf Horticultural	35	100.0	29	100.0	26	0.0
Extra Early Refugee	28	100.0	39	100.0	25	0.0
Full Measure	15	100.0	18	100.0	25	0.0
Sure Crop	15	100.0	39	100.0	15	0.0
Refugee Wax (caged control)	10	0.0	15	0.0	25	0.0
Late Varieties						
Refugee 100–1	29	100.0	25	100.0	25	4.1
Roger's Stringless Green Pod	25	100.0	45	100.0	15	20.0
Robust (resistant)	45	0.0	42	0.0	25	0.0
Refugee 1000–1 (caged control)	10	0.0	30	0.0	25	0.0

susceptible varieties except where caged to exclude insects. In sharp contrast those planted in August were relatively free from mosaic. In another like but larger plot, in 1926, the results were similar, with mosaic infection of 60 to 100 per cent on the two earlier plantings (June and July). Alongside these were plantings on two later dates of which that made August 1 showed small infection viz, 0 to 20 per cent, while that planted August 15 escaped entirely. The explanation seems to lie in the fact that the August plantings broke through the ground at a time when the insects had almost entirely disappeared, and hence they escaped infection.

Like results were obtained in 1927, when seeds from selected healthy plants of ten susceptible varieties were planted on seven successive dates in duplicate plots numbered I and II. The planting dates were: May 20–25, June 6, June 10, July 1, July 15, August 1, and August 15. The results are summarized in table 4. These findings indicate that when the seed is

TABLE 4.—*Further data on the relation of the date of planting to the amount of mosaic infection at the end of the season (1927 trials on ten susceptible varieties). (Note the escape from infection of those planted in May as well as those planted in August)*

Date of planting	Percentage of mosaic plants at end of season	
	Plot I	Plot III
May 20–25	0–20	0– 40
June 6–July 15	50–80	80–100
August 1–August 15	15–25	15– 60

planted early, the plants have a greater chance of escaping infection and suffer less damage from mosaic than when the seed is planted much later. Plants resulting from the May planting were vegetatively mature and in full blossom or podding stages when subjected to infection through insects. Plants subjected to infection at late stages in their growth, as was mentioned earlier, are less seriously affected by mosaic or may even fail to produce typical symptoms of the disease. The late-maturing varieties, however, owing to their longer growing and flowering periods, are hardly able to escape infection in the field and subsequent damage from mosaic, even when planted as early as May 20–25. These varieties, even though close to the flowering stage, are still growing actively when insects appear in July. The early-maturing varieties, on the other hand, are already in the blossoming or podding stages at this date. The amount of injury suffered by the early varieties, however, increases with the lateness in planting date.

While the percentage of infection was low on the August 1 and August 15 plantings, planting at these late dates is of no practical value under Madison climatic conditions, since most of the varieties would still be in the flowering or early podding stages in September when frosts may occur. Our results suggest, however, that when early or late commercial beans may be planted early in May, the amount of damage resulting from field infection may be reduced materially.

Such plantings, even though subjected to high insect transmission and showing 100 per cent infection later in the season, are less injured by mosaic, since they will have reached the flowering or podding stage before infection occurs. It is probable that in other regions, also, some advantage may be gained if planting dates can be so regulated as to prevent the period of maximum vegetative activity of the bean from coinciding with the period of maximum aphid activity.

SEED TRANSMISSION

Transmission of mosaic through seeds of legumes has been reported by Kendrick and Gardner (13) for soy bean, by McClintock (14) for lima bean, and by Dickson (5) for pea, red clover, alsike clover, and sweet pea; but bean mosaic provides, perhaps, the best known example of seed transmission of a virus disease. In their tests Reddick and Stewart (20) found that 50 per cent or more of the seeds from infected parents produced mosaic plants. Archibald (1) and others have likewise found that bean mosaic is transmitted through the seed. In the present investigations, a great variation in the amount of seed transmission was observed in different varieties of commercial beans, and the percentage of seed infection also has been found to vary with the stage of growth when infection of the parent plant occurred, as will be discussed later.

In tests made in 1926 and 1927 as high as 60 per cent of seed infection was found in seed of different late susceptible varieties on the market. Some of the seed lots from the same varieties, however, did not show such high infection. From table 5 it will be seen that, in general, the early varieties show much lower percentages of seed infection. The immune variety, Robust, and a few early varieties, showed no seed infection.

In these studies no evidence has been found that the relative vigor or viability of bean seeds is affected by the presence of the mosaic virus within them. Seed harvested at like stages of maturity appear to germinate equally well whether developed on mosaic or on healthy plants. It has been found that part of the seeds in a single pod may be mosaic-infected and part may be noninfected. In such case both infected and noninfected germinate with equal readiness and vigor. This fact that the presence of

TABLE 5.—*Percentage of seed infection in samples of commercial bean seed*

Variety	1926 test		1927 test	
	Total number of plants	Percentage mosaic	Total number of plants	Percentage mosaic
SNAP BEANS				
Early Varieties				
Giant Stringless Green Pod	215	3.5	280	0.0
Bountiful	236	4.0	270	0.0
Davis White Wax	205	0.0	270	1.5
Pencil Pod Wax	259	1.5	265	2.0
Tennessee Green Pod	195	1.0	280	4.3
Improved Golden Wax	140	0.0	280	0.0
Refugee Wax	300	10.0	280	5.0
Dwarf Horticultural	180	2.8	255	2.4
Wardwell Wax	150	5.0	125	3.0
Full Measure	235	0.0	270	3.0
Golden Wax	95	1.1	270	1.5
Sure Crop	125	0.0	270	0.0
Black Valentine	155	0.0	275	0.0
Late Varieties				
Roger's Stringless Green Pod	276	15.9	945	52.1
Refugee 1000–1	288	13.8	270	55.5
Hodson Wax	115	19.1	255	13.4
FIELD BEANS				
Early Marrow	242	15.0	250	30.0
Large Marrow	175	5.0	—	—
White Navy Pea	—	—	85	18.0
Mexican Bean	—	—	57	5.3
Robust	250	0.0	704	0.0

the virus within the seed does not influence germination has material significance as bearing on the seed transmission of the disease.

RELATION OF THE STAGE OF GROWTH OF THE PARENT PLANTS WHEN INFECTED TO THE AMOUNT OF SEED INFECTION

Our experiments have shown that the amount of seed infection is related to the stage of growth of the parent plant at which infection occurs (Table 1). Plants arising from infected seeds or healthy plants which became infected during early vegetative growth produced more infected seed than did those which became infected at later stages. The appearance of flowers marks the turning point in this respect, since plants infected after the

flowers were set produced almost no infected seed. These facts are illustrated diagrammatically in figure 7, which is based on the results obtained in tests of 1927.

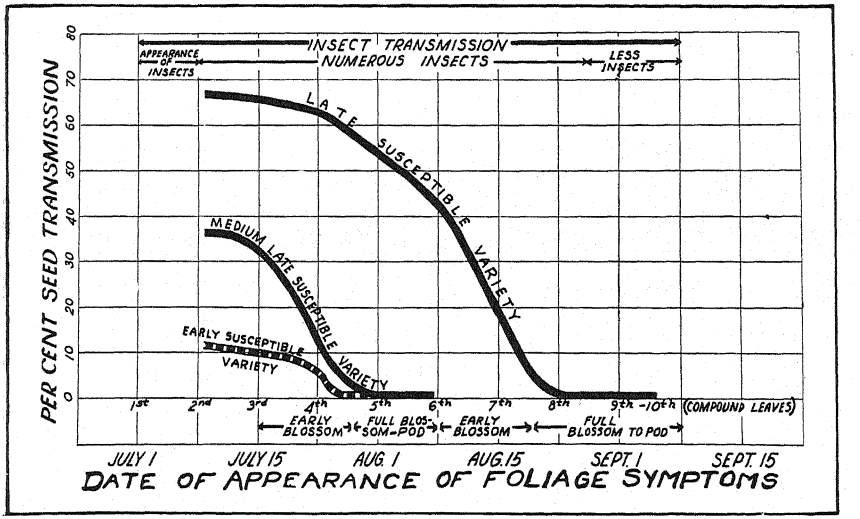


FIG. 7. Diagram illustrating the relation of the stage of growth of plants at which infection occurs to the percentage of mosaic transmission resulting in the seed. The date of appearance of foliage symptoms was used as an indication of the stage at which infection took place. The period of insect activity is indicated at the top of the diagram.

A wide difference was noted between the amount of seed infection occurring in the early and the late varieties. Early varieties infected during the second or third compound-leaf stage showed less seed infection than late varieties infected at the same stage. We believe this is due to the difference in relative stage of growth of the plants at the time of infection. The early varieties blossom and form pods at the third to fifth compound-leaf stage, so that plants infected at the second or third compound-leaf stage will be blossoming or podding when symptoms appear, and most or all of the seeds will escape infection. The late varieties, on the other hand, even if not infected until the fifth or seventh compound-leaf stage, will show symptoms long before the flowers are set; consequently a greater number of seeds will be exposed to infection. These facts may therefore explain why late varieties show relatively more seed transmission of mosaic than do early varieties.

RELATION OF THE STAGE OF MATURITY OF THE PODS TO THE AMOUNT OF SEED INFECTION

The results of a number of tests showed that, from the same mosaic plant, pods at different stages of maturity varied in the percentage of in-

fected seeds within them. This fact is illustrated diagrammatically in figure 8. The trials were made on the late susceptible Green Refugee

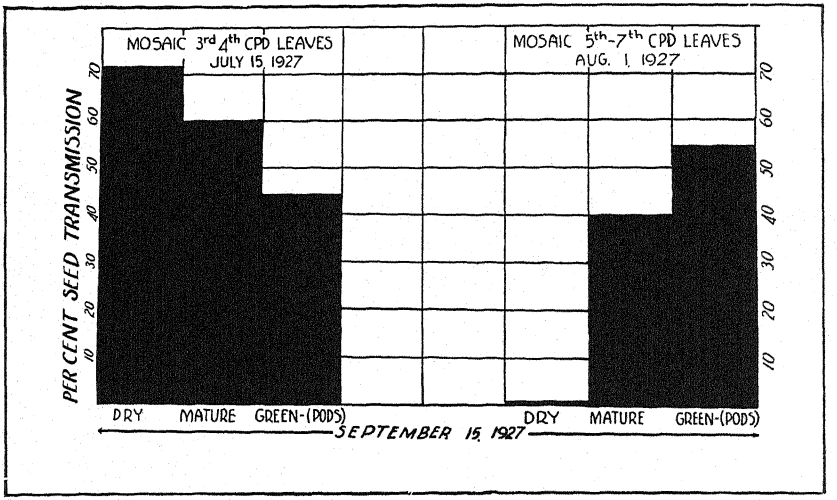


FIG. 8. Diagram illustrating the relation of the stage of maturity of the pods to the amount of seed infection. The pods were picked on September 15, 1927, and the seeds subsequently tested in the greenhouse (Roger's Stringless Green Pod).

variety. When the plants were infected early (third to fourth compound-leaf stage) a high percentage of infection was obtained from the older, dry, ripe pods; whereas, the pods set later, both mature and immature, showed a lower percentage of infected seeds. When the plants were infected later (fifth to seventh compound-leaf stage) there was little or no seed infection in the early-set pods, but the amount of seed infection increased greatly in the pods set later. When infection was further delayed to the full-podding stage, no seed infection was found in any of the pods. This last statement is, however, based on a test with only 10 plants.

No tests have been made with early varieties, but it is believed that these would give similar results, although the range of variation might be less because of the shorter growing and blossoming periods.

The position of the pods on the main stem did not appear to have any direct effect upon the amount of seed infection.

RELATION OF THE DATE OF PLANTING TO THE AMOUNT OF SEED INFECTION

Planting the seed at different dates influences not only the amount of mosaic occurring on the plants in the field but, also, the amount of seed infection in a given variety. Since, as shown above, the amount of seed infection is directly correlated with the developmental stage of the plants

when infection occurs, this is only to be expected. Our results indicate that beans planted early in May have a greater chance to escape infection through insects, and consequently infection of the seed, than do those planted later, i.e., between June 6 and July 16. Varieties planted in May will have reached the third or fourth compound-leaf stage by the beginning of July when they become liable to insect infection, while those planted later are exposed to infection as soon as they break through the soil. Since it takes about two weeks for symptoms to appear after infection, the plants in the May plantings, infected on or after July 1, will have already reached the blossoming or podding stage when symptoms appear and few or none of their seeds will become infected. Those planted in June or July are exposed to insect infection at much earlier stages and develop mosaic symptoms before the pods are set. Such plantings have, therefore, an increased chance of seed infection.

The most favorable time for planting depends upon the variety and the abundance of insects. In one test, early varieties planted between June 15 and July 15, in a field where insects were scarce, showed little or no seed infection at the end of the season. Where aphids are numerous and infection general, such varieties have increased chances of seed infection as the planting date is delayed. The late varieties, however, on account of their longer growing period, always yield a high percentage of infected seed, even though planted in May or June. Nevertheless, the amount of seed infection is lower than when the planting is done at a later date.

No explanation can yet be offered to account for the reduction in amount of seed infection in plants inoculated at later stages of growth.

CROSS-INOCULATION STUDIES

No success has been yet obtained in many attempts to transmit the virus of bean mosaic to other leguminous or nonleguminous hosts by various methods of inoculation, nor has any infection resulted when inoculations were made by the usual leaf-mutilation method from mosaic-infected leguminous or non-leguminous hosts to young, healthy bean plants. These results indicate, as suggested earlier, that the mosaic disease of the bean is, perhaps, specific to the bean plant, *Phaseolus vulgaris*.

This idea is consistent with our observations that the initial amount of mosaic development in a given bean field is directly correlated with the percentage of infected seed that is planted. The problem of mosaic control with bean mosaic is, therefore, much simpler than with those virus diseases where there may be various other neighboring host species.

RELATIVE SUSCEPTIBILITY OF THE BEAN PLANT TO MOSAIC INFECTION AT DIFFERENT STAGES OF GROWTH

Our experiments and observations indicate that susceptible varieties of bean may be readily infected by the virus of bean mosaic at any stage of development except that attempts to infect seedling plants in the cotyledonary or very early simple-leaf stage have thus far given negative results. The variety Robust has not yet been infected at any stage of its development, either artificially or when exposed to high insect transmission in the field. This is in accord with its reputation for immunity from mosaic. Nearly all the other commercial varieties of bean have been tested, however, and all these are readily infected and develop leaf symptoms in different degrees of severity.

SUGGESTIONS FOR THE CONTROL OF BEAN MOSAIC

The studies here reported were taken up with the object of extending our information, not only on the fundamental aspects of the disease, but also on the problem of control. Our knowledge of the disease is still so incomplete that further studies are necessary for the elucidation of many points relating to the disease and its prevention.

Since bean mosaic, so far as known, overwinters only in the seed and is spread from one locality to another through infected seed in commerce; since the spread in the field is, according to our evidence, accomplished largely if not entirely by aphids, and the extent of field infection is influenced not only by the abundance of these insect carriers but also by the number of plants originating from infected seed; and since the amount of seed transmission is related to the stage of growth when the plants are infected, the following methods of control are suggested as being in accord with the experimental results presented in this paper.

(1) *Production of mosaic-free seed should be accomplished:* (a) By selecting for seed purposes only healthy plants. Selection must be made before the symptoms of mosaic are masked. Plants with masked symptoms can, however, generally be recognized as diseased by examination of the lower leaves, which retain mosaic symptoms; (b) if such healthy plants cannot be obtained, then proceed by selecting plants infected late in the season, preferably those that were not infected until the early- or full-pod stages; (c) by establishing isolated seed plots. These should be planted with seed from carefully selected mosaic-free plants. If this is not available, then use should be made of seeds carrying very low percentages of infection. Such seed plots should be located at some distance from other bean fields and carefully watched with prompt roguing of mosaic plants continued until the blossom stage; (d) if no seed plot is maintained then

the general field from which seed is to come should be similarly rogued until the blossom stage; (e) by adjusting the date of planting, if possible, to bring the varieties to full vegetative growth and to the flowering stage before insects become active. Our trials have shown that, under the conditions of our experimental fields, nearly complete avoidance of seed infection may be attained by early planting of many excellent varieties.

(2) *Seed should be sown which is healthy or contains only a very low percentage of infection.* It appears to be entirely feasible to test lots of bean seed by growing representative samples in index plots, or in the greenhouse, to determine the percentage of infected seed in advance of the time for general field planting.

(3) *Resistant varieties should be used where practicable.* Robust is the best of these, if a white field bean is desired. In order to meet the needs with other types parent plant selections and hybridization should be encouraged.

(4) *Mosaic escape.* Attention should be given to the evidence that either early- or late-planted beans may escape insect infection. It is noteworthy also that the late-maturing varieties suffer most; therefore, attempts should be made to produce varieties maturing early enough to lessen infection, especially that reaching the seed, and which are otherwise satisfactory as to yield and quality.

SUMMARY

(1) Bean mosaic is coextensive with bean culture in the United States. It is one of the more serious diseases of the bean, since it is severe on certain leading varieties of both canning and field beans and is increasingly becoming a limiting factor in bean culture in certain sections of the country.

(2) The general symptoms and effects of the disease are described, including symptoms of plants infected during the season and plants originating from infected seed.

(3) Well-marked symptoms develop at 20–28° C. with an incubation period of 8 to 15 days; a partial masking occurs at 28–32° C., together with an increased incubation period; total masking occurs at 12–18° C.

(4) Inoculation and other experiments indicate that there is only one mosaic disease of the bean *Phaseolus vulgaris* L., which appears to be limited to this one host plant.

(5) The virus appears to be present in all aerial vegetative parts of mosaic plants. In the seed it apparently is limited to the embryo.

(6) No transmission of the disease has been obtained through the soil or through contact of roots or of aerial parts of mosaic and healthy plants.

(7) A leaf-mutilation method of inoculation has been developed which has yielded from 80 to 100 per cent infection in the majority of cases.

Inoculation through cut roots has given infection in a low percentage of cases. Soaking healthy seed, whether dormant or germinating, in infective juice gave no infection.

(8) The virus has been successfully transmitted by certain aphids: *Aphis rumicis* L., *Myzus persicae* Sulz., *Macrosiphum solanifolii* Ashm., and by a species of mealybug. Fifteen or more infected aphids per plant gave a higher percentage of infection than did fewer, although a single viruliferous individual of either *A. rumicis* or *Myzus persicae* was found to cause infection in some cases.

(9) The bean leaf hopper, *Empoasca fabae* Le B., and other species of insects tested for bean-mosaic transmission have thus far given negative results.

(10) Great variations in the prevalence of bean mosaic were observed in a given locality. The occurrence and amount of the disease in a field are chiefly determined by (a) the number of mosaic plants originating from infected seed which may serve as primary sources of inoculum; (b) the relative prevalence of aphids which may act as virus vectors.

(11) The amount of field infection and the extent of the injury from mosaic can be greatly reduced by planting early enough to permit the plants to go through their period of vegetative activity and reach the blossoming stage before aphids become abundant. The amount of injury increases with the delay in date of planting. The late varieties, however, owing to their longer growing period, cannot get through the season without being subjected to insect transmission, thus a higher percentage of infection and greater injury result than with the early varieties.

(12) Bean mosaic is usually transmitted through the seed and so far as known, is carried over from one season to another only in infected seed. Thus the spread from one locality to another is largely through infected seed in commerce.

(13) A wide difference in the amount of seed transmission has been observed between early and late varieties, as high as 60 per cent infection having been found in the commercial seed of late susceptible varieties.

(14) Seeds from plants infected during the current season do not all show mosaic infection. In an individual pod some seeds may be infected and others not. The percentage of seed infection is correlated with the stage of the plant when infection occurs and decreases as the plant approaches the blossoming period. Plants infected after the blossoms are set show no seed infection.

(15) The viability of the seed does not appear to be affected by the presence of the virus.

(16) The date of planting has been found to affect the amount of seed transmission in a given variety. Early varieties planted early are more likely to escape seed infection than late varieties. Both varieties, planted at Madison between May 20 and 25, showed less seed infection than those planted on June 10 or later. In general, commercial seed of early varieties shows less seed infection than that of the late varieties.

(17) Plants of susceptible varieties are apparently susceptible to mosaic infection at any stage of their development after the early seedling stage. It was not found possible to obtain infection of Robust, a resistant variety. All other commercial varieties tested were found to be susceptible to infection and showed varying degrees of severity of leaf symptoms.

(18) The methods of control suggested are: (a) Production of mosaic-free seed, (b) testing of seed lots in advance and planting only seeds that are free or nearly free from infection, (c) the development and use of varieties reaching maturity early enough to escape seed infection and still produce good yields, (d) the development and use of resistant varieties.

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CULTURES AND STRAINS OF THE STINKING SMUT OF WHEAT[1]

JESS R. KIENHOLZ AND F. D. HEALD

INTRODUCTION

Stinking smut is the most destructive disease of wheat in the Pacific Northwest. It not only causes direct losses by destroying the wheat kernel but also contaminates the sound grains to such an extent that during the period from 1923–24 to 1927–28 an average of 36 per cent of the cars coming to the terminal shipping points was given a smut dockage (10).

Prior to 1918, only *Tilletia tritici* (Bjerk.) Wint. was known to occur in the State of Washington. During 1919, an extensive survey of 631 fields was made of the wheat districts of Washington and only two cases were recorded of the appearance of *Tilletia levis* Kühn, the smooth-spore smut. These two specimens were found in Island and Lincoln counties.

Selection of resistant strains of wheat and the breeding of new varieties have been carried on continuously in this region with the result that several forms have been developed which are very resistant to or immune from the stinking smut that has been common to Washington. Besides Ridit, Albit, Martin, White Odessa, and others, certain strains of Turkey wheat could safely be sown without the regular seed treatment and a clean crop expected. These strains and varieties gave much promise for the future elimination of smutty wheat. These same varieties remained free from infection in the cereal nursery at Pullman, when inoculated with smut common to the Pullman district. Upon examination of the material from Moro, Oregon, a mixture of *T. tritici* and *T. levis* was found. Wheat growers became alarmed at this attack on their formerly resistant wheats and a number of samples from Washington were received at the college experiment station. Examination of these showed that both species of smut were present, but, as certain strains of Turkey wheat were known to be practically immune from the common form of *T. tritici*, the rough-spore species on these resistant varieties could only be explained by the introduction of a new form of the parasite.

Different varieties of wheat inoculated with collections of smut from various localities by Gaines, at Pullman, Washington, left little doubt as to the existence of new physiologic strains of the parasite in this region. In 1927 and 1928, quite an extensive survey of the wheat fields of eastern Washington was made to ascertain the distribution of the two species of

[1] Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper No. 161, College of Agriculture and Experiment Station, Pullman, Washington.

smut. This survey was carried out by the accumulation of warehouse samples of smutty wheat during 1927 and field collections in 1928. Although *T. tritici,* the rough-spore smut, still remains the predominating species of the State, the smooth-spore species (*T. levis*) was found to be present in all the main wheat-growing sections of eastern Washington as illustrated in figure 1.

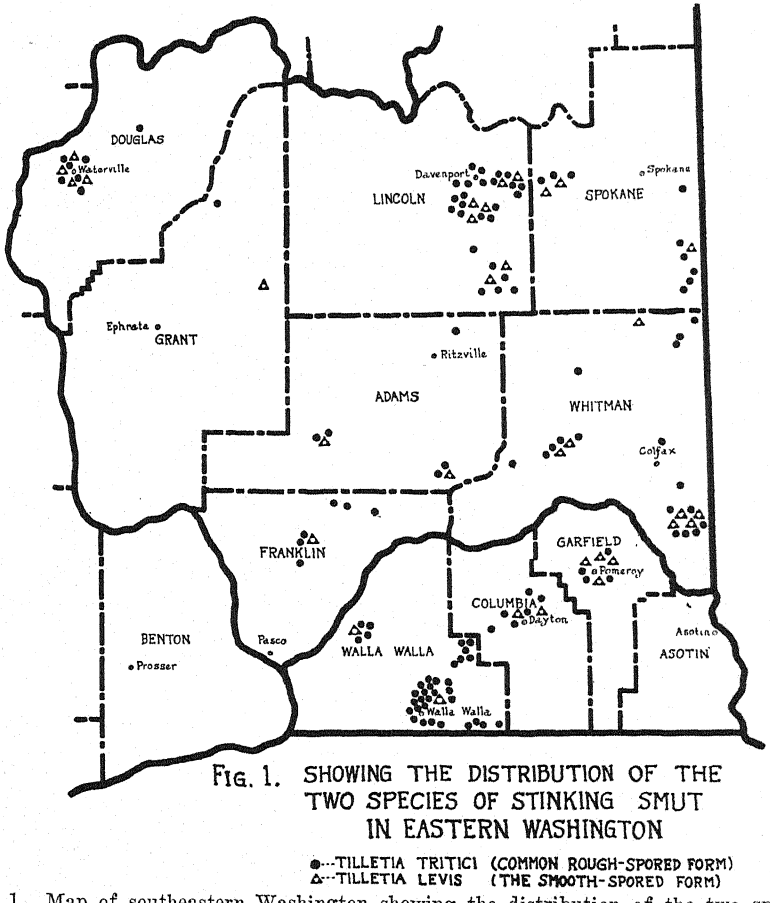


FIG. 1. Map of southeastern Washington showing the distribution of the two species of stinking smut.

The present work was undertaken to find a suitable solid medium on which to grow the smut and also to test the possibility of separating the physiologic strains by means of artificial cultures.

HISTORICAL SKETCH

The early work of Brefeld showed that the smuts could be grown on an artificial medium and be maintained through many generations. For this

purpose he used various nutrient solutions. After this pioneer work, little advance was made in culturing smuts until Potter (5), in 1914, published his results on growing the head smut of corn and sorghum on a solid medium. This gave promise of simplifying much of the technique in culturing smuts and also of making possible a more thorough study of their life cycles, which is sometimes quite difficult with the cultures on liquid media.

Sartoris (9) gives the first account of growing the wheat stinking smut on a solid medium in 1924. He found that a 2 per cent malt-extract agar was favorable for the germination and growth of the smut spores. These were found to germinate in two to three days and, after a period of two or three weeks, a mycelial mat formed on the surface of the medium.

Reed, Stephens, Stakman and Rodenhiser, Gaines, and others have definitely shown that physiologic strains of the organisms causing stinking smut now exist in this country. In the Pacific Northwest, these forms are thought to have been introduced since the World War and no doubt are responsible for much of the increased smut present in cars shipped from this region within the past few years. Reed (6) has separated four distinct races of *T. levis* and six of *T. tritici* by their degree of infection on certain wheat varieties; Rodenhiser (7), three of *T. levis* and two of *T. tritici;* while Gaines (3) separates four forms of *T. levis* and three of *T. tritici* in his tests at Pullman. Some wheat varieties remain very resistant to all forms of the smut tried but the majority are attacked rather severely by at least one form. The work on physiologic specialization of stinking smut, however, has hardly started.

A recent publication by Rodenhiser (8) gives some interesting results on the separation of physiologic strains of cereal smuts, but strains of stinking smut based on cultural characters have been omitted.

METHODS

The process of culturing the stinking smut of wheat was early found to divide naturally into two main phases: 1, germination; and 2, growth.

Germination

The desired medium was first poured into petri dishes and allowed to solidify. The end of an unbroken smut ball was then cut off and about one-fourth to one-half the contents emptied into a 10 cc. sterile water-blank by rotating the smut ball between the fingers. (Very little contamination was experienced by this method, especially on media lacking the concentrated food materials.) With a sterile pipette, about $\frac{1}{2}$ cc. of this spore suspension was introduced on the surface of the medium and distributed

by agitating the petri dish. The cultures were then ready to be incubated at the proper temperatures.

Single chlamydospores were easily obtained by the dry-needle method (4), substituting a fine-drawn glass hair for the steel needle.

Growth

After small, visible, white "colonies" formed on the surface of the medium (usually about two weeks, although this was found to vary considerably), they were carefully picked out with a sterile needle or fine-pointed scalpel and transferred to another medium more suitable for the growth of the organism. It is usually advisable to grow these cultures in flasks to retain sufficient moisture in the medium and to lessen the possibility of contamination because of the length of time required for growth. The medium in the flasks was usually slanted slightly because of the tendency of some cultures to form an abundance of sporidia which might easily fall off and be a means of differentiation later. Better growth will usually take place when only enough agar is used to properly solidify the medium, the extremely hard media apparently withholding much of the necessary moisture and slowing down the spread of the colony.

When the cultures once become well established in test tubes or small flasks, the quantity of inoculum may be materially increased by introducing sterile water into them and transferring a portion of this sporidial suspension to a larger surface of the medium in a large flask and incubating the cultures at the proper temperature.

RESULTS

Germination

Attempts to follow the methods of germination outlined by Sartoris (9) usually gave poor results. Most of the collections of smut used by the writer failed to produce sporidia when planted on 2 per cent malt-extract agar. Various media were then tried in an attempt to find one that would give more uniform results. The optimum temperature for germination was found to be between 18° and 20° C., but many spores germinated through quite a range of temperature. Good results were obtained at temperatures near 10° C., although the process of colony formation was considerably retarded, while some germination and subsequent growth even took place at room temperature (25° C.). The latter temperature usually caused the media to dry out, however, before the sporidia would form. The various media used and their effect upon the germination of the smut spores follow:

1. *2 per cent malt-extract agar or gelatine.* Different collections of the smut varied greatly in their ability to germinate on this medium, the major-

ity of those tried failing to produce sporidia. In these cases, if the chlamydospore germinated at all, the proycelium would rupture before sporidial formation, allowing the contents of the cell to exude in a granular form, or a short promycelium would develop which degenerated before attaining much length. In this latter case, the form of the promycelium usually became irregular and warty, in contrast to the smooth, straight walls of the usual type.

2. *Carrot-2 per cent dextrose agar.* This medium which Potter found to be suited to the germination and growth of the head smut of corn also gave poor results with the stinking smut of wheat. Most of the spores would germinate, but these usually produced a thickened and much-branched promycelium which failed to produce sporidia. The contents in many of the tip cells of this branched system degenerated, being successively cut off by a septum (Fig. 2, A.). Sporidia formed very rarely, but when they did occur these would then form the colony growth in the usual manner.

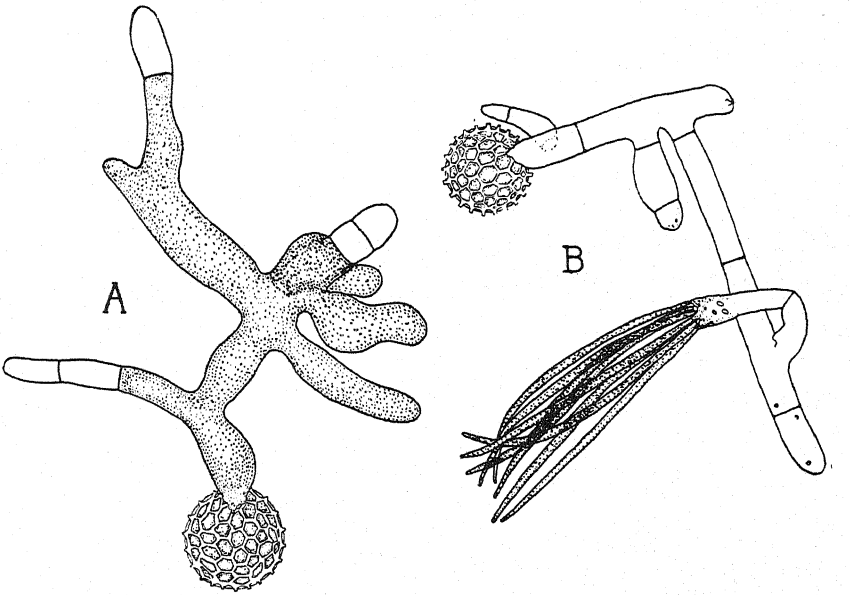


FIG. 2. Germination of spores of *Tilletia tritici:* A, after eleven days on carrot-2 per cent dextrose agar; B, after twelve days on soil-extract agar plus 10 per cent kaolin

3. *Potato-2 per cent dextrose agar.* The ability of the smut spores to germinate on this medium varied greatly also. Some plates produced many of the small white colonies after a few weeks, while others practically failed to even germinate. It was not definitely determined whether certain strains

of the organisms exhibited any constant difference in this respect, but some indication of this seemed possible.

4. *25 per cent cod-liver oil agar.* Colonies of the organism formed on this medium and the majority of the spores germinated, but the process of sporidial formation was retarded at least one week. The promycelium became longer and more vacuolate and the resulting sporidia fewer in number or smaller. It was not considered useful for germination.

5. *Plain agar.* Quite uniform germination took place when plain agar was used, and this proved to be one of the best solid media of those tried for the germination of the spores. A few difficulties were encountered which remain inexplicable, but, generally, a high degree of success was obtained. It was found that the medium containing at least 20 grams of agar per liter was more satisfactory than one containing less because of the tendency for the moisture to collect around the petri-dish cover, which shut off some of the supply of essential oxygen. The harder medium tended to retain much of this moisture.

It is advisable to add 3 or 4 grams of common salt per liter to the medium when only a few spores are to be introduced, as this prevents much of the "exploding" of the cell contents, apparently by keeping the cell-sap concentration in the promycelium more nearly equal to that of the surrounding medium. The exact coefficient was not determined, however. Many of the spores germinate in two days, while others require several. After about two weeks the small colonies form and are then ready to be transplanted to the medium more suitable for growth.

6. *Soil-extract agar.* Seventy-five grams of garden soil was placed within a funnel on filter-paper, and 500 cc. of boiling water was allowed to filter through. To this, 20 grams of previously prepared agar was added and the volume made up to 1 liter.

This medium proved the most useful for germinating the stinking smut spores. It seems to furnish the essential constituents of the soil where the spores naturally germinate, although the extract of peat soils would probbly be unfavorable for germination because of the large amount of soluble organic material it contains. Clay soil extracts are not as good as those from loam soils but they do furnish a satisfactory medium.

The spores germinate within a few days, according to their individual peculiarities, and form the small colonies in about two weeks. At least 90 per cent germination has resulted in most cases, although certain cultures were not so prolific. This was probably a result largely of the collected moisture around the petri-dish covers, however, which prevented the entrance of air, rather than being a fault of the medium. No special tests were made to determine how long the smut spores would retain their vitality,

but one collection five years old gave over 50 per cent germination on this medium.

Some chemicals were added in small amounts to this medium, as calcium nitrate, kaolin, and others. Most of these when added in small quantities caused an "over-stimulation," and the promycelium was often queerly branched, as shown in figure 2, B. The sporidia were rarely formed under these conditions until the promycelium had become much branched and septate and the cell contents nearly depleted.

Single-spore isolations

The results with single-spore isolations have been very discouraging. From some hundred single spores isolated, only two spores formed the sporidia and subsequent colonies. Spores from the same smut ball when introduced onto the medium in larger numbers, however, gave a high percentage of germination and growth. Sartoris (9) states that his cultures were started from single spores on the 2 per cent malt-extract agar. The spores send out the promycelium which soon bursts, and the granular cell contents escape, leaving an empty cell, which finally disappears.

The two spores which formed the sporidia and colonies from the single spores developed on the soil-extract agar. It is possible that, if the proper dilution of salts is present in the medium, much of this difficulty would be overcome, but further experiments are necessary to determine this point.

As previously stated (see Methods), these spores were easily obtained by the dry-needle method. The spores were "puffed" onto the slide from small pieces of paper upon which part of a smut ball had been crushed or broken. Contamination was rarely experienced with this method.

Growth

After colony formation in petri dishes, it was found advisable to transfer the cultures to 50 cc. flasks (or other suitable containers) containing about 25 cc. of medium. The medium in the petri dishes dries so rapidly that growth in the plate cultures is soon arrested. The optimum temperature for the growth lies near 20° C., although fair growth results through a range of temperatures from 10° to 40° C.

Cultures of stinking smut differ from most of the smuts that have been cultured previously, mainly in the length of time they require to reach an appreciable size and the lower temperatures needed for optimum growth. A month usually was necessary after their transference from the petri dishes to attain a diameter of one-half inch. This characteristic varies considerably with the various strains of the organisms, the above figure being more nearly an average growth. The slower-growing forms of *T. tritici*

were smaller than the corresponding forms of *T. levis,* while the faster-growing types of both species exhibited similar relations in culture, as shown in figure 3.

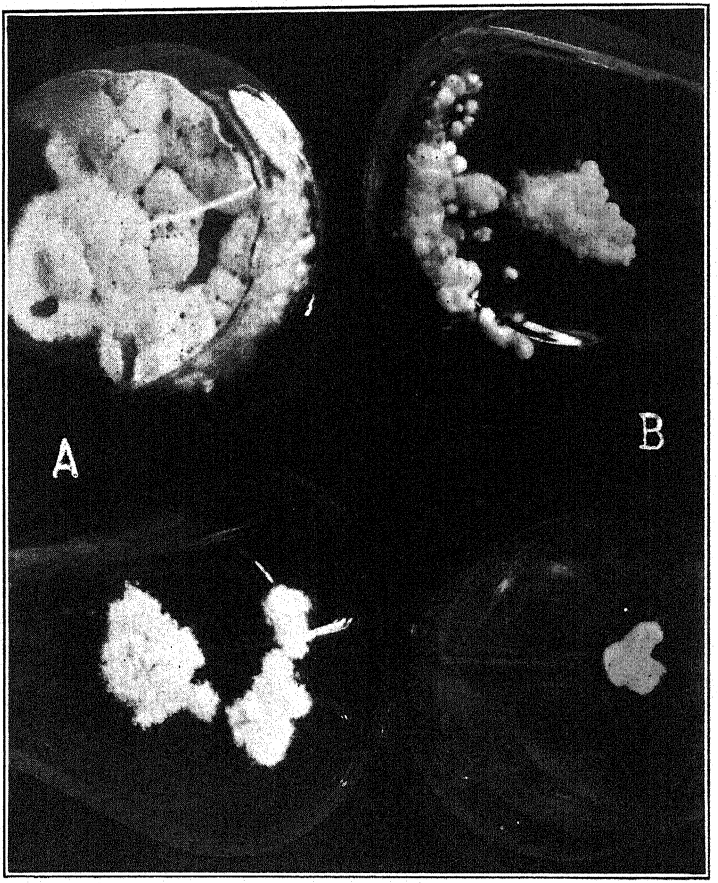


FIG. 3. Thirty-day-old flask cultures on potato-4 per cent sucrose agar of the fastest and slowest growing strains: A, *Tilletia levis;* B, *T. tritici.*

Growth was found to consist of two distinct forms according to the type of medium upon which the cultures were planted. Although all cultures grew similarly for a period of time, all media containing added sugars generally caused the growth to become heaped after a period of several weeks, a character not found in media to which no sugars were added. This heaped type of growth, which is especially noticeable in cultures of *T. tritici* more often than the other species, is composed of compact mycelial threads, pseudoparenchymatous in nature, and may often reach a thickness of

nearly an inch in the older cultures of some types. The growth on the media containing no added sugars remained flat, and, if lifted from the surface of the medium with a needle, appeared much like the membrane of an egg and had a similar consistency (Fig. 4).



FIG. 4. Flask cultures showing the effect of nonsugar medium and sugar-containing medium on type of growth: A, on wheat-seedling agar; B, on potato-sucrose agar.

Many of the cultures exhibited the phenomenon of forcible discharge, already noted by previous workers (1), this being evident by the scattered colonies forming in all directions from the original planting on a slanted medium.

Some of the media tried and the growth characteristics exhibited by the same strain of smut will be listed to give a general idea of the type of growth that may be expected with other media.

Nonsugar media

1. *Wheat-extract agar.* This medium was made by presoaking 60 grams of wheat seed over night in water, boiling in a cheesecloth container for a half hour the following day, and draining off the extract. This was generally filtered through cotton, after which 14 grams of agar per liter was added.

Growth consists of a flat surface type of rather thin consistency but quite favorable for rapid development. Media made from the extracts of five varieties of wheat, namely, Ridit, Turkey, Jenkins Club, Triplet, and Hybrid 128, caused no noticeable variation of growth with the same transfer of smut. It is concluded that, if any chemical or enzymatic resistance is present in these varieties, these principles are broken down during sterilization processes.

2. *Wheat-seedling agar.* Two hundred and fifty grams of young wheat seedlings were allowed to soak in warm water for several hours, the mass then being pressed out through cheesecloth. Eighteen grams of previously prepared agar was added to this and the volume made up to 1 liter.

The medium caused a flat colony of rapid growth which was of a more powdery consistency than the growth on the other nonsugar media tried. Because of the difficulties entailed with its making, however, it is not considered practical for general use.

3. *Starch solution plus agar.* This medium produced a very thin, slow growth and was not considered of value in this work.[2]

4. *Heavy oatmeal agar.*—(Unstrained.) This constitutes the best medium of this general class, especially upon which to carry stock cultures in test tubes. Growth was slightly more rapid; a flat, fairly powdery colony developed; and subcultures were more easily made than from most other media. The oatmeal juice used by the writer was thick enough to be sufficiently solidified with 6 grams of agar per liter. (100 grams of rolled oats cooked in 500 cc. of water and squeezed through cheesecloth.)

Media with added sugars

1. *Two per cent malt-extract agar.* Good growth takes place on this medium, but it is not quite so rapid as that on some of the others. The surface of the culture tends to become somewhat more loosely arranged, giving it a more cottony appearance.

2. *Carrot-2 per cent dextrose agar.* The growth is similar to that on malt-extract agar.

3. *Potato-2 per cent dextrose agar.* The culture tends to become somewhat heaped or more ruffled than in the two preceding media, with the surface growth more compact, giving the culture a glossy or waxy appearance. Growth is rapid and the medium is considered among the best tried.

4. *Potato-4 per cent sucrose agar.* It was found that sucrose used in the same proportions as dextrose was slightly more favorable for growth. This medium was generally adopted for the remaining experiments because of its differentiating properties. More rapid growth resulted on this medium than on any other of this general class.

5. *Soil-extract agar plus 2 per cent dextrose.* Fair growth developed, but the medium is inferior to most of the others for experimental work.

6. *Synthetic asparaginate agar.*[3] A growth similar to that on potato-dextrose agar results but develops somewhat slower.

[2] See Harshberger, A Textbook of Mycology and Plant Pathology, p. 610. 1917.

[3] See Waksman, S. A. Principles of Soil Microbiology, p. 16. Williams & Wilkins Co., 1927.

7. *Sartoris's synthetic agar.* (9) This medium causes a growth about equal to potato-dextrose agar but of a slightly different type. The surface of the culture tends to become more velvety at first or slimy later and less furrowed (in this strain) and appears generally more loosely arranged.

Several original synthetic media were tried, but, as results with them have been no better than with those already mentioned they will not be given.

Results with form collections

It must be stated at the outset that the work on physiological forms is not conclusive. Only a beginning could be made in one year's time, and, since much preliminary work was necessary with both the culturing and, later, the individual strains, perhaps this point can be more fully appreciated.

The collections of smut used for these experiments were largely obtained from two sources; forms of *T. levis* being those used by Dr. E. F. Gaines in his field experiments at Pullman, Washington, while *T. tritici* was mostly taken at random from the collections made jointly by Marion Griffiths Zehner, of the United States Department of Agriculture, and the writer, in eastern Washington. One sample sent from the University of Halle-Salle, Germany, and the specimen of bunt collected by Gaines on rye also were used.

Growth of the cultures showed very little difference on the nonsugar media, and even some of those containing added sugars failed to differentiate the various collections of smut grown on them. Potato-4 per cent sucrose agar, however, was found very useful for this purpose and was generally adopted, although cultures on other media were also made simultaneously for comparison. Of the media tried, those having the best differentiating properties for wheat smut were, potato-4 per cent sucrose agar, potato-2 per cent dextrose agar, oatmeal-3 per cent dextrose agar, and Sartoris's synthetic medium, in the order named.

Preliminary cultures of the above collections yielded five distinct forms of each species (Figs. 5 and 6), but conflicting changes have appeared in subsequent transfers. It appears that with each successive transfer the growth of the colony usually takes place more rapidly, tends to become more heaped or mycelioid, or some other modification results. How long these changes would continue to occur is not known, nor was it determined whether the original transfers from the petri dishes would show constant characters upon starting their growth. Dickinson (2) found in his work on the oat smut that eight single sporidial isolations from two different promycelia gave as many different types of cultures, so it becomes a matter

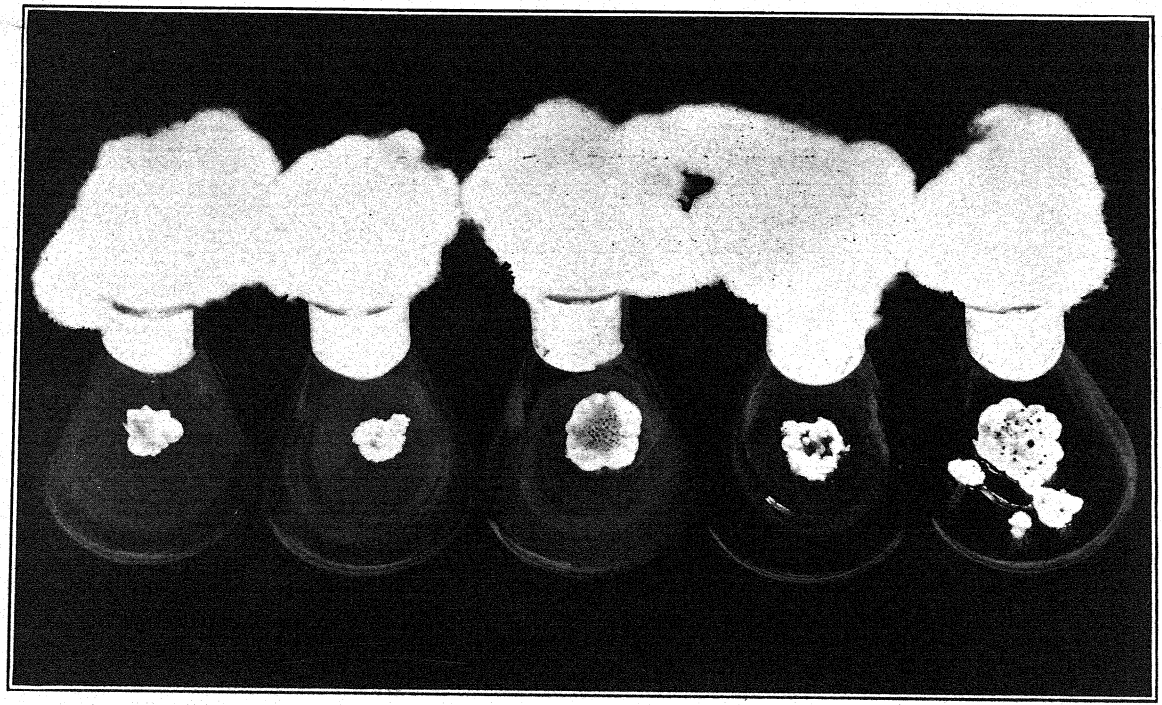


FIG. 5. Thirty-day-old cultures of five different collections of *Tilletia levis* on potato-4 per cent sucrose agar. Nos. 6, 2, 10, 7, and 1, from left to right. (See tabulation.)

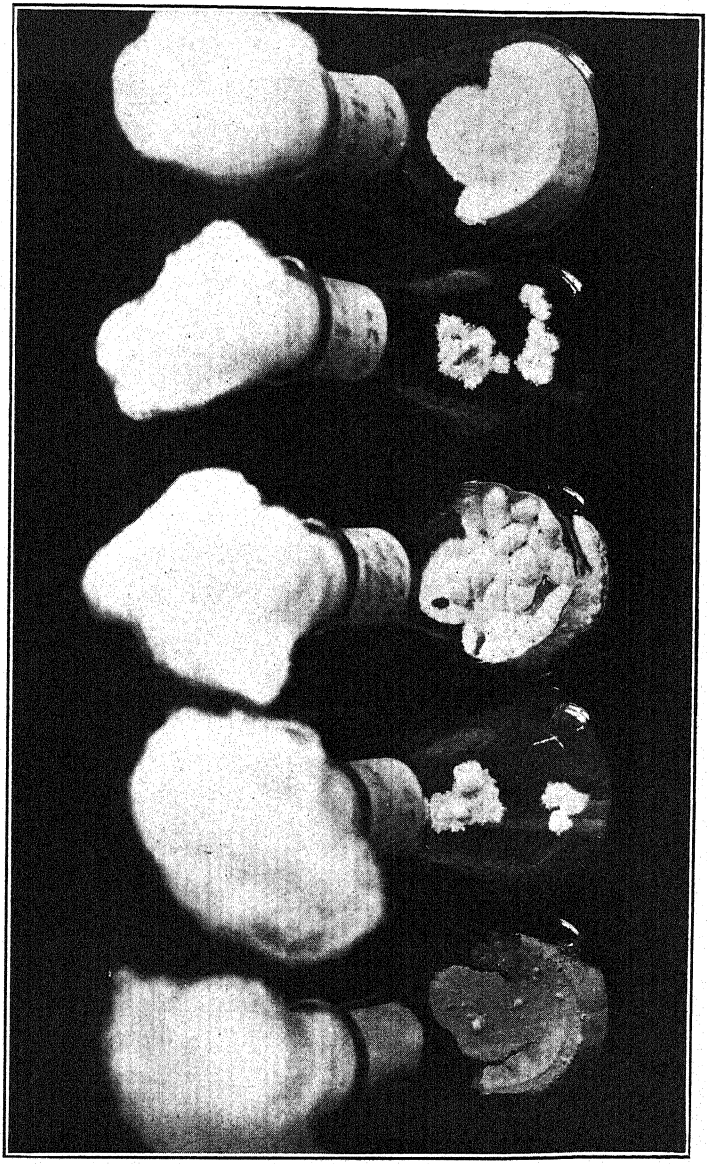


FIG. 6. Thirty-day-old cultures from five different collections of *Tilletia tritici* on potato-4 per cent sucrose agar. Nos. 7, 10, 4, 2, 6, from left to right. (See tabulation.)

of speculation when one deals with cultures of stinking smut which were not even derived from single chlamydospores. Several transfers of one form would vary slightly when handled under identical conditions, although in some strains this variation appeared to be more pronounced than in others. The results obtained with these preliminary cultures may give some idea of the difficulty encountered in trying to segregate physiologic forms by means of cultural methods.

Cultures one month old grown on potato-4 per cent sucrose agar and incubated at a temperature of about 20° C. (in a specially constructed compartment under an ice box which was cooled by means of the drip water) were used for comparison. Due to the lack of space, only a limited number of cultures could be grown at once, and there was also a chance for slight fluctuations in temperature.

Since the different strains isolated did not show a constancy of cultural characters in the transfers following the original series (Figs. 5 and 6), it will not be of particular value to give the detailed description of their behavior. A tabular outline of the cultural characters of the five strains of *T. levis* and the five strains of *T. tritici* will, however, be presented in order that a better idea may be gained of the variations (Tables 1 and 2). Under each culture number the first description applies to the first series of cultures, the characters being based on 30-day-old cultures on potato-4 per cent sucrose agar; while the second description is based on transfers made later from these original cultures and grown to equal age on the same kind of medium and under the same temperature conditions. It may be noted that there were variations in rapidity of growth, color and compactness of the cultures, color of the medium, topography of the cultures, and sporidial production.

SPORE CHARACTERS OF THE FORM COLLECTIONS

The spores of the smuts used in these experiments were carefully examined for any minute characters which might be used to separate the strains by microscopic methods alone. The smooth-spore strains showed many irregularities in this respect even when taken from the same portion of the smut ball. Some were quite round, others oblong, or extremely irregular in outline. No constant difference could be found between the spores of the various collections.

Spore characters in the rough-spore strains were more pronounced. The spores from the various collections varied from those having coarsely reticulate walls to those in which it was difficult to determine whether the reticulations were present on the epispore or the inner membrane. One could hardly keep from believing that some of the spores were the result

TABLE 1.—*Summary of the characters of the original cultures and subsequent transfers of Tilletia levis on potato-4 per cent sucrose agar at the age of one month (Fig. 5)*

Culture No.	Size in inches	Color of culture	Consistency	Colony character	Color of medium	Source of smut and host variety
L 6[a]	2 x 2	Dirty white	Powdery	Flat, rapid grower; thin margin; discharged sporidia	Dark sorghum-brown	Lind, Wash. Khapli
	Do.	Center pure white	Velvety powdery	Flat except central raised drops. No sporidia discharged	Sorghum-brown	
L 2	¾ x ½	White	Powdery cottony knobs	Raised ⅛″; discharged sporidia; margin irregular but definite	Unchanged	Waterville, Wash. Albit
	1 x ⅞	Pure white	Cottony	Raised ¼″; surface furrowed; margin smooth; no discharged sporidia	Do	
L 10	1¼ x 1¼	White	Cottony	Raised ¼″; many raised knobs; furrows filled with droplets	Unchanged	Lind, Wash. Hybrid 143
	1⅛ x 1⅛	Do	Do	Do	Slate-drab	
L 7	¾ x ½	Brownish	Powdery	Flat; discharged sporidia; irregular margin	Ecru-drab	Lind, Wash. Khapli
	1 x ⅝	Pure white	Cottony	Raised ¼″; surface furrowed; no sporidia discharged	Unchanged	
L 1	2 x 2	White	Cottony	Flat; mycelioid margin	Light ecru-drab	Lind, Wash. Hybrid 128
	1½ x ⅞	Variable	Powdery	Flat; zonated buff, gray and white	Do	

[a] The upper spaces are the original cultures; lower spaces, the subsequent transfers.

TABLE 2.—*Summary of the characters of the original cultures and subsequent transfers of Tilletia tritici on potato-4 per cent sucrose agar at the age of one month (Fig. 6)*

Culture No.	Size in inches	Color of culture	Consistency	Colony character	Color of medium	Source of smut and host variety
T 7[a]	½ x ½	Pure white	Cottony	Raised surface ⅛″; furrowed margin smooth and definite	Tinged ecru-drab	Fairfield, Wash. Ridit
	⅝ x ⅝	Do	Do	Do.	Lighter	
T 10	½ x ½	Pale flesh	Compact powdery	Flat, definite margin; slow growth	Unchanged	Halle-Salle, Germany
	Do	Dirty white	Cottony	Raised ⅛″; surface furrowed margin smooth and definite	Do	
T 4	⅝ x ⅝	Pure white	Compact cottony	Raised ¼″; surface furrowed bearing colorless droplets	Unchanged	Ritzville, Wash. Turkey
	1 x 1	Do	Do	Same with the exception of the droplets	Do	
T 2	½ x ½	Muddy; white border	Cottony	Raised ⅛″; central part muddy, white border growing over it	Tinged ecru-drab	Milton, Oregon Federation
	1 x 1	White	Do	Raised ⅛″ surface slightly furrowed, all white	Unchanged	
T 6	⅝ x ⅝	White	Cottony	Raised ⅛″; furrowed, bearing brown droplets. Sporidia discharged	Sorghum-brown	Reardan, Wash. Turkey
	Do	Do	Do	Similar but without droplets; no sporidia discharged	Light sorghum-brown	

[a] The upper spaces are the original cultures; lower spaces, the subsequent transfers.

of crosses between the two species. The differences in spore characters of these various collections, however, did not show the degree of constancy in the cultures obtained from them.

Generally, it may be said that the spores of *T. tritici* appeared circular in outline and showed the characteristic wall markings, while those of *T. levis* tended to be more oblong or irregular. It was with extreme rarity that both species or what appeared to be a mixture of forms occurred in the same smut ball.

INOCULATIONS USING PURE CULTURES

Since it would be of value in the study of the smut problem to be able to make inoculations by the use of pure cultures, some preliminary tests were made. Four pure cultures were used: Two of *T. levis* (see L 1 and 7 in Table 1) and two of *T. tritici* (see T 10 and 2 in Table 2). The cultures were increased by introducing sterile water into cultures in small Erlenmeyer flasks, similar to those shown in figures 5 and 6, and making a suspension of sporidia. A few cubic centimeters of this suspension were then added to the surface of the medimu (potato-4 per cent sucrose agar) in 2,000 cc. flasks, which soon gave a copious growth completely covering the surface of the agar. Two varieties of wheat, Bluestem and Khapli, known to be susceptible to the strains of *T. levis*, and two varieties, Jenkins Club and Spring Federation, known to be susceptible to the two strains of *T. tritici*, were treated with formaldehyde and allowed to dry. Suspensions were made from the large cultures and taken immediately to the field and used at once for inoculating the seed samples which were planted while still moist. Rod rows were planted in duplicate and one half of each planting was drenched with the remaining sporidial suspension before covering to give an added opportunity for infection. There was no smut whatever from any of the culture inoculations or in the formaldehyde treated checks, while adjacent plantings using ground smut gave an average of 53 per cent of smut. These results are in agreement with the findings of Sartoris (9). No explanation for failure to obtain infections with cultures can be offered unless there is a requirement of mingling of two different cultures.

SUMMARY

1. The smooth-spore stinking smut (*T. levis*) has become generally distributed over the wheat sections of eastern Washington and readily attacks many of the wheat varieties resistant to the rough-spore species (*T. tritici*).

2. It is apparent that physiologic forms of this species and also the rough-spore smut, common to this section, likewise exist and greatly complicate control measures against these serious parasites.

3. Culturing of the organisms is greatly facilitated by dividing the process into stages: (1) Germination; (2) growth after germination.

4. Plain agar or soil-extract agar are the most suitable media of those tried for germinating the smut spores.

5. Media containing added sugars usually cause a heaped type of growth to form, while in those containing no added sugars the cultures remain flat.

6. Potato-4 per cent sucrose agar was found to be the medium best suited to differentiating the form collections of smut.

7. Different collections of smut exhibited various types of growth, but these differences were not constant in subsequent transfers; hence, it was not possible to segregate physiologic strains by cultural characters alone.

8. Spore characters were not correlated with the differences exhibited by these forms in culture.

9. Inoculations made under field conditions using pure cultures of *T. levis* and *T. tritici* were unsuccessful, as not a single smutted head resulted.

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GRAY LEAF SPOT OF TOMATO CAUSED BY STEMPHYLIUM SOLANI, SP. NOV.

GEORGE F. WEBER

In the summers of 1924 and 1925 the writer observed numerous small spots on the leaves of declining tomato plants in the vicinity of Gainesville, Florida. At that time no indication of a sporulating parasite could be detected with a hand lens on either surface of the leaf. Similar spots were observed again on tomatoes in the field in the spring of 1926 in Manatee County. Specimens were collected and laboratory examination revealed spores on the lesions on both surfaces of the leaves. Further observations within the past three years showed that the disease was more or less general on tomatoes in various stages of development from the seed bed through the harvesting period. The disease was found in destructive form in seed beds in the vicinity of Palmetto. Later in the season a survey showed it widespread in the county, and in several instances it was causing heavy losses. A specific 30-acre field at Terra Ceia was a total loss because of this disease. Infection had occurred early in the seed bed and was carried to the field on the plants. No marketable fruit was obtained from this field. The disease was observed in destructive form on the lower east coast of Florida during the season of 1929, where it caused more damage to tomatoes that year than any other disease. Since the disease has been collected in these three widely separated sections, it would be only natural to suspect that it has a more or less general distribution in Florida. From the economical viewpoint it is of considerable importance both in the seed bed and in the field.

DESCRIPTION OF THE DISEASE.

So far, both in the seed bed and in the field, the disease has been found limited almost entirely to the leaf blades. Under very favorable conditions occasional lesions have been observed on the petioles, but no infection has been found on stems, branches, or fruit.

Serious infections in the seed bed results in marked defoliation of the plants without conspicuous yellowing. In the field, however, the yellowing of the lower leaves after the spots develop is a conspicuous symptom. The disease advances rapidly from the lower leaves to the growing tips of the branches. The affected leaves die rapidly, become brown, and are shed. In severe cases all of the leaves are shed, except small infected leaves at the growing tips of the branches, where the development of new leaves and the destruction of them by the fungus have apparently come to a balance.

Gray leaf spot first appears as minute brownish black specks. In light infections there may be one to several spots on a leaflet, or in severe infection they may be so thick that half the entire surface of the leaf blade is occupied by these tiny spots less than one millimeter in diameter. These spots are occasionally marginal and in such places are somewhat elongated or irregular in outline. On other portions of the leaf the spots are more or less circular, slightly sunken when observed from either surface of the leaf, and variously scattered over the blade without any apparent restriction by the veins. The individual spots show simultaneously on both surfaces of the leaf and are surrounded by a narrow halo band when viewed with transmitted light. With reflected light, there is a sharp contrast between the brownish spot and the normal green of the leaf blade. The smallest spots, barely visible, measuring $\frac{1}{10}$ to $\frac{1}{5}$ mm. in diameter, do not usually show this sharp line of demarcation between the diseased and healthy green tissue. At this time there is no apparent yellowing of the leaf. As the spots enlarge the central killed area changes from a brownish black to a grayish brown and the color contrast between the healthy and diseased tissue becomes pronounced. The whole spot becomes somewhat shiny or glazed. These changes continue until the spots attain a size of about 2 mm. in diameter. By this time, there is, in most cases, a definite yellow area ap-

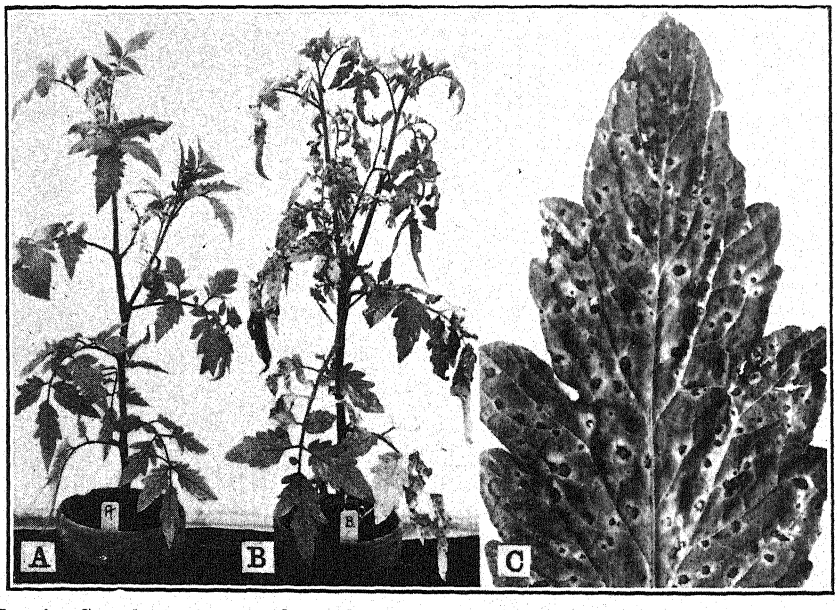


FIG. 1. Greenhouse tomato plants showing: A, noninoculated check plant. B, inoculated plant showing gray-spot disease after six days. C, tomato leaf showing natural infection by *Stemphylium solani*.

parent around the larger spots (Fig. 1, C). Spots are seldom found with greater diameter than 2 mm., except on the very oldest leaves near the base of old plants. Under such conditions, individual spots may attain a diameter of 4 mm. or more. As the centers of the spots dry out they often crack from side to side and form various patterns. The central area may break away entirely from the leaf, leaving holes of various diameters and with jagged edges in the center of the individual spots, which gives a shot-hole appearance to the leaf when viewed in its entirety. It is often in this stage that the yellowing of the entire leaflet becomes most conspicuous, especially if the infection is severe. This condition is followed in quick succession by wilting and drooping and eventually dying and shedding of the leaves. The spores have not been found on the diseased areas until the upper surface of the spot shows the grayish brown color and the glazed or shiny surface. They are readily found after that, appearing first on the erect conidiophores in the central portion of the spot on the lower surface of the leaf blade. As the spot ages and eventually cracks and falls away, the fruiting area increases and the conidiophores are found almost on the blackish margin and on both surfaces of the leaf. Occasionally, on the older, somewhat yellowish leaves that are more or less shaded at the base of the plant and appear to be subnormal in their functions the spots coalesce, involve, and kill large areas of the leaf blades, which become brown and dried. The individual spots on these leaves have been found as large as $\frac{1}{2}$ cm. in diameter, but such development is not common. In contrast with other leaf spots of tomatoes, caused by species of Alternaria, Macrosporium, and Phoma, the Stemphylium spots are small and more regular and evenly distributed and do not enlarge rapidly. They are almost circular and of a uniform grayish brown over the killed area, and show no concentric zonation as is characteristic of spots formed by species of the three genera mentioned above. Spots caused by *Cladosporium* sp. differ from gray spot in that they appear as large yellow blotches on the upper surface of the leaf immediately above the infection on the lower surface, and the spots caused by *Septoria* sp. differ in that they usually show a light colored central area or frog-eye spot that is more or less speckled with black pycnidia.

PATHOGENICITY.

Isolations of the fungus were made from typical leaf lesions collected in the field by plating our surface-disinfected leaf sections, by pouring dilution plates of a water suspension of spores, and by mechanically removing single spores from the lesions and planting them on poured potato-dextrose-agar plates. Germination of the spores and growth of the mycelium were rapid and conidia were produced in profusion.

The conidia produced from single-spore cultures were used in water suspensions for inoculation experiments. The spore suspension was atomized on leaf blades, petioles, and stems of potted tomato plants 8 to 10 inches high. After they were inoculated the plants were placed in a moist chamber for 36 hours and then placed on a greenhouse bench. Check plants were treated in a similar manner except that water was atomized on them instead of the spore suspension. After 48 hours signs of infection were visible on the inoculated plants and the spots developed rapidly during the next 4 days. Six days after inoculation some of the lower heavily infected leaves began to yellow, droop, shrivel (Fig. 1, B), and finally shed. Eventually all leaves that were inoculated were shed, leaving only several small leaves at the tip, which had developed since the inoculum was applied. Isolations were made from the lesions produced by inoculations and the characters of the fungus obtained were indistinguishable from those of transfers of the original culture used for the inoculations. Inoculations were made with the reisolated fungus and symptoms of the disease were produced on the tomato leaves similar to the symptoms resulting from the first artificial inoculation. Further inoculations were made with conidia from leaves collected in the field and with conidia produced in pure culture to compare symptoms of the disease produced. The disease produced in each case was essentially the same. Other cultivated hosts determined by artificial inoculation (4) are *Capsicum annuum, Solanum melongena* and *Physalis pubescens.*

As previously mentioned, this disease is distinct from other leaf spots found on the leaves of tomatoes. The fungus is different in most essential points from any other fungus reported as parasitic on tomato plants. Furthermore, none of the available descriptions of parasites producing muriform spores seems to fit the species. Because of the existing varying descriptions of the genera Alternaria, Macrosporium and Stemphylium (2, 3), one is in doubt as to the correct position of this fungus. In this case, however, the writer will follow Elliott (1) and place the fungus in the genus Stemphylium and, since it is essentially different from known species, will describe it as a new species.

Stemphylium solani, sp. nov.

Hyphae variously branched, septate, and intercellular. A dense growth is made on various media with a conspicuous and abundant production of conidia. Conidiophores dark, septate, slightly larger than the infertile hyphae, rigid, 130–200 μ x 4–7 μ, swollen tips and with irregularly shaped bases. Conidia produced acrogenously on simple septate conidiophores on the host and on potato-dextrose agar after 6–10 days. In older cultures conidiophores become branched and conidia appear pleurogenous; primary conidia often germinate *in situ,* produced 1 to 8 secondary conidia and occa-

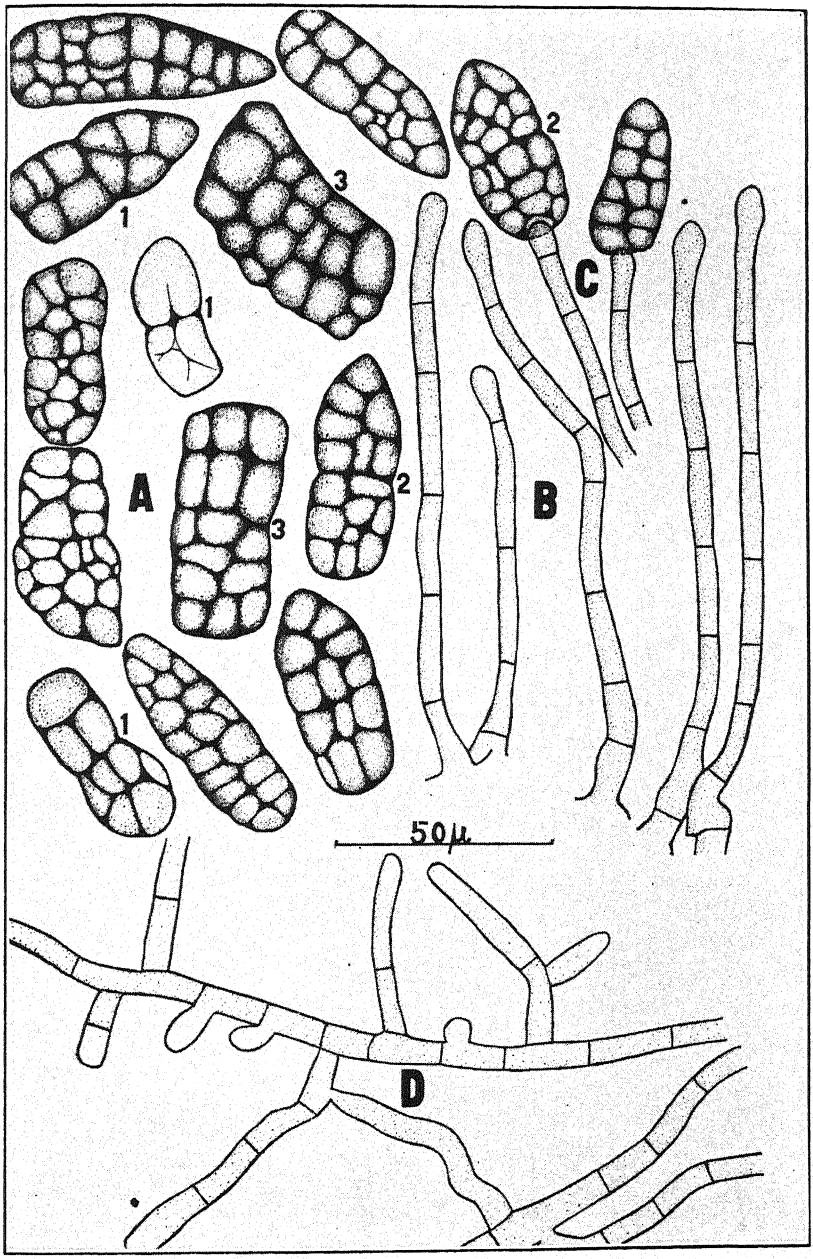


FIG. 2. Camera-lucida drawings of *Stemphylium solani* showing: A, conidia from host, (1) young, (2) mature (typical), (3) old. B, Conidiophores from host. C, Conidiophores and attached conidia from culture. D, Mycelium from culture.

sionally tertiary conidia slightly smaller than the secondary conidia. Conidia muriform, fuscous to very dark, oblong-rectangular, more or less rounded at ends or one end often somewhat pointed, several longitudinal septae, constricted near medial septum, other septations more or less irregular, transverse septae several to many, depending on age of conidia. Wall smooth when young, slightly reticulate after maturity, 50 per cent or more of conidia 45–50 μ x 20–23 μ, average 48.08 μ x 22.43 μ (Fig. 2). Ascigerous stage of fungus not known. Isolated from leaves of Globe, Marglobe, and Earliana tomatoes.

Type material in Florida Agricultural Experiment Station Herbarium.

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THE EFFECT OF GRAFTING ON RESISTANCE AND SUSCEPTIBILITY OF TOMATOES TO FUSARIUM WILT

CURTIS MAY

The literature on the subject of grafting in relation to the passage of elaborated substances from scion to stock or *vice versa* indicates that in some plants certain materials may be transferred without alteration.

Colin and Franquet (1) found that inulin was changed to sucrose when it passed the graft layer between artichoke and sunflower. Grafe and Linsbauer (2) demonstrated that nicotine passed from *Nicotiana tabacum* to *N. affinis,* increasing from nil in the ungrafted *N. affinis* to 1.67 per cent in grafted plants. Javillier (3) reported that atropine passed from a belladonna scion to a potato stock, and Laurent (4) found that the same transfer took place when belladonna was grafted on tomato.

Leach (5) has reviewed some of the recent work regarding the effect of grafting on disease resistance in plants. Roach (6) concludes from grafting experiments that the reaction of any potato tissue to wart disease is innate in the individual cells and is not transferable except by cell division.

Most of the work on hydrogen-ion concentration and buffer action, tannins, and carbohydrates in relation to disease resistance supports this conclusion. Or, as Leach (5) points out, the factor or factors conferring immunity or resistance, if transferable, lose their identity in the cells receiving them.

In the experiments reported here a parasite which causes a systemic disease is involved. If some specific transferable substance is responsible for resistance or suceptibility, it seems that it should be demonstrated in this type of disease by reciprocal grafts of resistant and susceptible varieties.

Two varieties of tomato, Bonny Best, very susceptible to wilt, and Norton, a resistant variety, were used in the experiments.

When the plants were from four to six inches tall they were approach grafted. The unions were wrapped with raffia. The wounds healed in about ten days, after which the root system of one and the top of the other of a pair of plants were severed. A number of reciprocal unions were secured. In about two weeks some of the grafted plants were transferred from the sterile soil in which they were growing to soil inoculated with *Fusarium lycopersici* Sacc. At the same time nongrafted plants of each variety were planted in the contaminated soil. Checks of both varieties, grafted and nongrafted, were left in the sterile soil, and a temperature of approximately 30° C. was maintained.

The "Norton scion-Bonny Best-stock" plants began to show symptoms of wilt 26 to 30 days after they were planted in the inoculated soil. A week to ten days later the leaves were badly wilted and the plants were obviously dying. The plants of the reciprocal combination grew larger and showed no signs of disease at this time. Nongrafted Bonny Best plants developed wilt at the same time and nongrafted Norton plants remained healthy. Both grafted and nongrafted check plants, grown in sterilized soil, developed no disease.

The root systems of these two plants were very different. The Bonny Best roots were badly infected and not so extensive as the healthy and abundant Norton roots.

The vascular bundles of the Bonny Best stock were discolored up to the point of union with the Norton. Here the fungus had crossed into the Norton and progressed a little way up the stem. However, the discoloration in the Norton bundles was not so pronounced as in the Bonny Best bundles immediately below them, and it disappeared about four inches above the graft. The vascular system of the Norton stock-Bonny Best-scion combination was apparently healthy.

It is evident that the Bonny Best scion did not materially alter the resistant property of the Norton stock. Likewise, the Norton scion did not confer resistance to the Bonny Best stock. Moreover, after infection took place in the Bonny Best stock, the fungus was able to cross the graft layer and pass into the tissues of the Norton section of the plant. However, the entire plant was distinctly subnormal. This may account, in part at least, for the growth of the fungus in the resistant scion. In addition, the resistance of the Norton variety to Fusarium wilt is relative. Under some unfavorable growing conditions or at the end of the season this variety becomes diseased.

In another experiment a plant of Norton and one of Bonny Best, grown in sterile soil, were grafted about 3 inches above the ground line. After union was complete the two plants were transferred to Fusarium-infected soil, where they grew high (30° C.). After three weeks the Bonny Best top began to show signs of wilt, while the Norton was quite healthy. A week later the Bonny Best top had entirely wilted down. The Norton top was then showing the first symptoms of wilt. On dissection, the Bonny Best showed vascular discoloration throughout its entire length. The Norton had discolored bundles above the graft, but not below. On cutting cross sections through the region of the graft it was evident that the infection came up through the Bonny Best side and passed from it into the Norton at the point of union.

Field and greenhouse experience has demonstrated that wilt-resistant varieties of tomatoes become infected with the wilt pathogene near the end

of the growing season. In the work reported here it did not gain entrance through the roots of the resistant variety. However, once established in the vascular system of the susceptibility variety, it was able to enter and grow in the vascular bundles of the resistant one. The question of localization of resistance in certain cells at once presents itself.

The experiments indicate that the cause of resistance or susceptibility of tomatoes to Fusarium wilt either is not transferable or loses its identity in the cells which receive it.

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VARIETAL SUSCEPTIBILITY OF THE PEONY TO BOTRYTIS PAEONIAE

ROGER WINTERS

During the season of 1929, dusting experiments on peonies were made at the Cornell University Test Gardens in an effort to control the Botrytis blight, *Botrytis paeoniae* Oud. During the course of these experiments it was noticed that some varieties appeared more susceptible to the disease than others. At the suggestion of Professor H. H. Whetzel, notes on varietal susceptibility were taken. These were taken during the last week in June, while the plants were in bloom. Four empirical degrees of susceptibility were recognized: Resistant, moderately resistant, susceptible, and very susceptible. Plants showing a total of not more than three infections on leaves and flowers were classed as resistant; plants showing not more than six infections were classed as moderately resistant; those showing more than six infections, but not so seriously affected as to render the flowers a complete loss from the commercial standpoint, were classed as susceptible; and those so seriously affected that the flowers were commercially useless were classed as very susceptible.

The peony plot in the Test Gardens contains a large number of varieties, but only two plants (or occasionally four) of any given variety were planted side by side. Thus most of the data given below are based on observation of but two plants. However, the two plants of each variety invariably showed comparable degrees of susceptibility, and, furthermore, it was not uncommon to find a very susceptible variety growing practically in contact with a resistant variety, which indicates that the amount of infection was dependent on varietal susceptibility and not on local conditions. The data presented cover a single year's observations only. With the above facts in mind, the following grouping of varieties according to their susceptibility appears to be worth recording:

RESISTANT

Akalu
Argus
Arthemise
Attraction
Avalanche
Balliol
Baroness Schroeder
Baron James Rothschild
Black Prince
Cavalleria Rusticana
Chalice
Christine Gowdy
Christine Ritcher
Comte de Nanteuil
Dorothy
Dorothy Echling
Dorothy E. Kibby
Ella Wheeler Wilcox
Eucharis
Eureka
Fragrans
Fulgida
General Bertrand
General Cavaignac
Gloire de Chenonceaux
Glorious
Glory of Somerset
Gretchen
Griff Thomas
Hermes
Ho-gioku
Iten-shikai
King of England
Kumagaye
Lady Bellew
Lady Mayoress

L'étincelante
Lord Salisbury
Luetta Pfeiffer
Madame Lemoinier
Madame Schmidt
Maud L. Richardson
Meisonnier
Monsieur Boucharlataine
Mrs. Gwyn-Lewis
Mr. L. van Leenwen
Old Silvertip
Petite Renée
Plutarch
Princess Ellen
Purpurea Superba
Queen Wilhelmina
Red Bird
Ruigegno
Sarah Bernhardt
Speedwell
Sweet Home
Yeso

MODERATELY RESISTANT

Admiral Dewey
Agnes Mary Kelway
Albâtre
Albert Crousse
Alfred de Musset
American Beauty
Archie Brand
Asa Gray
Augustin d'Hour
Aunt Ellen
Béranger
Bullock
Carnea Elegans
Carnot
Charles Binder
Charles Verdier
Charlotte Cushman
Clarisse
Comet
Conqueror
Couronne d'Or
Daubenton
Daybreak
Dorchester
Dorothy Kelway
Duchess of Portland
Duke of Devonshire
Edwin Forrest
Emile Lemoine
Enchantment
Eternal City
Etta
Faust
Favorite
Festiva Maxima
Flambeau
Frances Shaylor
Graziella
Grizzel Muir
Gypsy
Henry Avery
Hon. Mrs. Portman
Innocence
John Fraser
June Day
Jupiter: Calot
Kelway's Queen
La Coquette
Lady Alexandra Duff
Lady Somerset
La Fraicheur
Lake of Silver
La Perle
La Sublime
La Tulipe
La Vestale
L'étincelante: Dessert
Lord Lytton
Louis van Houtte
Mabel L. Franklin
Madame Coste
Madame de Guerle
Madame de Vatry
Mademoiselle Gaillant
Mafeking
Mary L. Hollis
Masterpiece
Mathilde de Roseneck
Mazie Terry
Monsieur Paillet
Monsieur Pasteur
Muchelny
Norfolk
Octavie Demay
Pallas
Phoebe Cary
Pierre Duchartre
Pink Enchantress
Princess Maud
Queen of Beauty
Rauenthal
Rhoda
Rubicunda
Ruth Brand
Simonne Chevalier
Sir Robert Gresly
Snowflake
Sosthenes
Sully Prudhomme
Torquemada
Triumphata
Trojan
Venus
Victoria
Ville de Nancy
Waterloo
Welcome Guest

SUSCEPTIBLE

Adam Bede
Agnes Barr
Amalthea
Armand Rousseau
Bertha
Camille Calot
Canariensis
Carlotta Grisi
Carnea Triumphans
Caul
Chrysanthemiflora
Comte de Cussy
Comte de Paris
Countess of Clancarty
Daniel d'Albert
Delachei
Duc de Cazes
Duc de Wellington
Eastern Beauty
Edmond Lebon
Etienne Méchin
Frances Shaylor
General Grant
Grandiflora
Jules Calot
Lady Beresford
Lutetiana
Madame de Verneville
Madame Emile Galle
Madame Hutin
Magnifica
Marie Lemoine
Marquise d'Ivry
Mathilde Méchin
Meadowvale
Monsieur Chevreul
Mrs. Lowe
Myrtle
Pottsi
Princess Beatrice
Pulcherrima
Queen's Perfection

Roem de Boskoop
Sappho
Sea Foam
Snowball: Hollis
Souvenir de Gaspard Calot
Strasbourg
Sunrise
Thomas S. Ware
Torch
Triomphe du Nord
Turana
Vicomtesse de Belleval
Virginie
Virgo Maria
Viscountess Folkestone
Whitleyi

VERY SUSCEPTIBLE

Antoine Porteau
Armandine Méchin
Assmanshausen
Belle of France
Charles Toche
General Bedeau
Grandiflora Lutescens
Irma
Lutea Plenissima
Nivea Plenissima
Paradise
Territorial
Victoire Modeste

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A CONTROL OF ANGULAR LEAF SPOT OF TOBACCO BY SPRAYING IN THE FIELD

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The frequent occurrence of angular leaf spot in what is known as the new belt or coastal-plain section of North Carolina has given this disease an importance which probably is greater than that of any other disease affecting tobacco in the coastal-plain section. Although it is the experience of the growers, in substantiation of the observations of plant-disease investigators, that some varieties are more subject to the attacks of the causal organism[1] than others, angular leaf spot continues to cause damage and at times produces a nearly complete destruction of tobacco in many tobacco fields. In addition to the varietal relationship of the disease, it has been observed by the writer since 1927 that rainfall greatly influences the prevalence of angular leaf spot, especially the weather conditions for a period of approximately one month after the crop is set in the field.

In 1929 weather conditions were such that angular leaf spot of tobacco appeared in some fields during the early part of June, or shortly after the plants were set, and by the middle or latter part of June the disease was rather prevalent in sections. It generally has been found that the disease originates in the plant bed. Consequently, spraying the plants in the bed with Bordeaux mixture has been recommended frequently by plant pathologists, in addition to sterilizing the seed and practicing other means of sanitation. On many occasions, however, a rapid spread of the disease takes place in the field apparently from a few infected plants, where weather conditions are favorable, as during a rainy spell.

An early outbreak of the disease combined with weather conditions favorable for infection made it appear that the summer of 1929 would be a favorable one for observing the effects of various spray materials on the control of angular leaf spot when applied to tobacco in the field. Demonstration plots were therefore arranged in a total of four tobacco fields, two in Hertford and two in Bertie counties. Two sprays were used in each instance; namely, Bordeaux mixture 4–5–50, using hydrated lime; and a mixture of zinc and hydrated lime (4–4–50) similar to that recently used in various parts of the United States for the control of the bacterial spot (*Bacterium pruni*) of peaches.

Three plots were laid off in each field, consisting of two sprayed plots and a check plot. The first applications were made during the period from June 11 to 13 when the plants were scarcely knee-high. The second

[1] *Bacterium angulatum.*

was made between June 24 and 27, when only a few of the plants were reaching the topping or blossoming stage. The nonsprayed plots were located between the two sprayed plots.

Leaf counts were made on July 12, approximately seventeen days after the final spray, and about one week before the first leaves were harvested from the most advanced plants. In preparing the spray ordinary laundry soap was used as a spreader at the rate of one pound to 50 gallons. This type of spreader was used in preference to calcium caseinate, since a spreader of the latter type has a tendency to increase spray residue on foliage, an objectionable feature in the case of tobacco leaves. Due to the type of spreader employed and also as a result of the rapid growth of the leaves, only a trace of spray material was observed on an occasional leaf at the time of harvest.

As indicated in the table, the angular leaf-spot disease, although bacterial in nature, was effectively controlled by both the Bordeaux and the zinc-lime sprays. Of the two sprays, Bordeaux mixture was the most effective. No burning resulted from either spray except in two of the plots where a compressed-air sprayer was used. This was not equipped with an agitator for mixing the spray, hence some burning of the leaves resulted from that portion of the spray coming from the bottom of the tank. Where the spraying was done with a machine equipped with an agitator this injury was eliminated.

Location of field	Treatment	Leaf spot heavy	Leaf spot light	Leaves sound
		Per cent	Per cent	Per cent
Eskew Minton farm, Hertford County, N. C.	Bordeaux mixture	0	31.2	68.8
	No treatment	2	58.7	39.3
	Zinc-lime	1	34.6	64.4
R. D. Sessoms farm, Windsor, N. C.	Bordeaux mixture	0	18.4	81.6
	No treatment	17.5	47.9	34.6
	Zinc-lime	9.3	43.2	47.5
Wallace Britt farm, Harrelsville, N. C.	Bordeaux mixture	0	27.9	72.1
	No treatment	0	62.	38.
	Zinc-lime	0	42.	58.
R. C. Mason farm, Hertford County, N. C.	Bordeaux mixture	.8	46.4	52.8
	No treatment	3.2	44.4	52.4
	Zinc-lime	3	41.9	55.1

In three of the tests a very noticeable improvement in the condition of the sprayed plants with reference to freedom from angular leaf spot was apparent in the field. This is indicated in the table, since in the first three

tests listed the plots sprayed with Bordeaux-mixture contained 29, 47, and 34 per cent, respectively, more sound leaves than adjoining nonsprayed plots. Bordeaux-mixture generally was more effective than the zinc-lime mixture. In the fourth test, on the Mason farm, the second application of spray was made just before a heavy rain. This may account for the less effective control of angular leaf spot. In all of the tests, however, the improvement of leaf quality was more than sufficient to offset the expense of labor and materials employed with spraying. The 1929 observations indicate that the sprays were applied at the time best designed to most effectively prevent the development and spread of the disease. The first application, made when the plants were small, required only about one-half the quantity of spray required for applying the second spray a few days prior to topping time.

Due to the increasing prevalence and the serious nature of the angular leaf spot disease in some localities, spraying the plants in the field with Bordeaux or zinc-lime spray should prove profitable, particularly where such highly susceptible varieties as the Cash and Adcock are grown. The observations in North Carolina indicate that angular leaf spot is the greatest limiting factor in growing these otherwise high-quality and desirable types.

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FRANK GETCHELL O'DONNELL, 1896–1928[1]

N. REX HUNT

Frank Getchell O'Donnell was born October 20, 1896; married Mary Alberson in December, 1923; received the B. E. degree from Schuylkill Seminary in 1917 and the B. S. degree in plant pathology from Pennsylvania State College in 1920. He was a graduate student of George Washington University and the University of Maryland. In 1920 and at various times previous to his graduation he was a field assistant in potato investigations with the Pennsylvania Agricultural Experiment Station. In April, 1920, he was appointed field assistant with the Federal Horticultural Board, later becoming plant quarantine inspector and soon afterward assistant pathologist, which position he held at the time of his death, January 11, 1928. His death was due to tuberculosis, following pneumonia and pleurisy, brought on by exposure during a storm in the winter of 1924–25, while en route to the University of Maryland, where he was carrying graduate work in biochemistry and plant pathology preparatory to studying the physiology of fungi.

He was a member of A. A. A. S., Virginia Academy of Sciences, American Phytopathological Society, American Society of Bacteriologists, Ecological Society of America, Botanical Society of Washington, Alpha Zeta, and Phi Kappa Phi. He was joint author of "Steam and Chemical Soil Disinfection with Special Reference to Potato Wart," Journal of Agricultural Research 1: 301–363, August 15, 1925; author of "Printing Plate Cultures," Phytopathology 12: 53–54, January, 1922; and "Hot Water Bulb Tank; Cheap Cooker for Hybridizer," Florists' Review 60: 31–32, June 16, 1927. Unfortunately his experimental work, which was being done on a large scale, was, as a whole, unfinished and the results were not published.

Such are the prosaic details of a brief biography, but what manner of man was he? He came from a gifted and long-lived family, and, expecting a long life, was building for the future on a broad foundation. His father, a store proprietor, piano teacher, and successful composer, quit music and has been a Bell Telephone city manager for twenty-five years. As promotions came the family moved from city to city, and young O'Donnell

[1] Read before the Botanical Society of Washington, December 4, 1928.

proceeded to make himself at home on and in the water for miles around, collecting all sorts of plants and animals, using his mother's cupboard, sewing basket, and closets for snake houses and such and filling the living room with aquaria. During his high-school days he cleared $600.00 a year selling fancy fish and aquatics. He also dabbled in chemistry and electricity, took up machine shop practice and wood working, and acted as "trouble shooter" for the telephone company.

In college he had no fastidious fish to feed and took to feeding fungi instead. At the time he quit potato-wart work to undertake seed disinfection studies he was carrying 1,200 cultures of organisms from soil given various treatments. In this and in some of his seed-disinfection work it took literally thousands of petri dishes to keep him satisfied. He had an uncanny knack of getting a pure culture of anything he wanted, from any medium, regardless of its nature or condition. As a pastime he grew hundreds of cultures of various fungi on different media and checked the resulting modifications of color, growth, and spore forms. He also experimented rather extensively on methods of preserving pure cultures in tiny packets and had a high degree of success in preserving such cultures for periods several times as long as any published records for them. The varying actions of such long dormant cultures when again given a square meal were a source of great interest and amusement to him. None of this work was ever prepared for publication.

Mr. O'Donnell was an indefatigable worker, often working half through the night. Most of the articles covered by the 2,000 entries in the bibliography used in the potato-wart work were read at night. He had thousands of bulletins and articles summarized and cross indexed in his private library.

He disdained to run experiments on a small scale, thinking nothing of personally counting 20,000 seeds to set up a series of experiments, such series often being repeated several times. He was a stickler for extreme accuracy. He was ingenious in modifying or creating apparatus to meet his needs. Most of his work was done in testing or proving worthless or inadequate numerous proposed or possible seed treatments. He spent much overtime for several months qualifying as an X-ray operator in order to test recommended seed disinfection by X-rays. He built modified vacuum disinfection chambers and tested numerous dangerous war gases. His pure-culture transfer chamber has been copied by several Government offices and other organizations. His records would have furnished material for several important articles if he had not insisted on exhausting all possibilities of each method and testing and retesting the results before committing himself in a publication.

With his great store of accurate and often first-hand information on many subjects, always gladly furnished to any one interested, it is little wonder that Mr. O'Donnell was constantly asked for help on many and diverse problems. He was busy until ten o'clock of the night he died.

His death was a loss to science because of what he might have accomplished had he been permitted to build on that foundation of thorough preparation in all allied sciences, skill in developing and using apparatus and methods, superaccuracy in all details, and limitless patience and perseverance.

THE CONTROL OF CERTAIN FRUIT DISEASES WITH FLOTATION SULPHURS

M. A. SMITH[1]

In the United States nearly all the native sulphur is mined by melting it with steam in the rocks in which it occurs and forcing it to the surface. This sulphur, which has a high degree of purity, is suitable for the manufacture of sulphuric acid and lime sulphur. When ground to sufficient fineness it may be used for fungicidal sprays or dusts.

Flowers of sulphur, the fine powder formed by the condensation of sulphur vapor, has been used extensively as a fungicide. However, due to improved machinery, it is now possible to secure a uniformly fine ground natural sulphur that is as efficient fungicidally as flowers of sulphur.

When ground sulphur or flowers of sulphur is added to pure water it floats on the surface because it does not become wet. By reducing the surface tension of the water with a suitable agent, it becomes possible to wet the sulphur particles and thereby secure a suspension which may be employed as a spray. Wettable sulphurs are now widely used in the United States. They may be safely applied to many types of foliage and, if sufficiently fine, are satisfactory fungicides.

With the introduction of lime sulphur in 1908 in Oregon, a very efficient fungicidal spray was secured. It has remained one of the standard sprays since its first introduction. Owing to the fact that it may cause serious injury under certain climatic conditions, there is a demand for a spray material which combines the high toxicity of lime sulphur with the safety of ground wettable sulphur.

In view of the importance of the degree of fineness of the sulphur particle, it is interesting to note that many attempts have been made to utilize a colloidal form of sulphur as a spray material. Ramsay and Cooke (5) have proposed the precipitation of lime sulphur by sulphuric acid. Young (9) added sulphuric acid to a concentrated solution of sodium thiosulphate by which means sulphur dioxide and free sulphur were produced. Other methods of preparing colloidal sulphurs have been proposed by various investigators. The preparation of colloidal forms of sulphur

[1] This project was made possible with funds supplied by the Koppers Company, of Pittsburgh, Pennsylvania, and administered by the Crop Protection Institute. The writer wishes to express his appreciation to Dr. H. W. Anderson, under whose direction these studies were made, and to other members of the Institute committee who have offered suggestions during the course of the work; and to the Department of Horticulture of the Illinois Agricultural Experiment Station for its cooperation in making available extensive facilities.

to be used on a large scale has not yet been found to be practicable from the standpoint of cost and labor involved in their preparation.

Recently there has been developed in the manufactured gas industry a process, known as liquid purification, by which large quantities of pure precipitated sulphur are obtained. This is known as flotation sulphur and is obtained through a process described by Sperr (7, 8) and Sauchelli (6).

When flotation sulphur leaves the filter press it contains about 50 per cent of water and certain impurities of the gas, namely, sodium thiosulphate and sodium thiocyanate. They are water-soluble, however, and may be easily removed by washing, leaving a pure sulphur cake. There are three types of flotation sulphur known, respectively, as Thylox, Ferrox, and Gray. Thylox sulphur is recovered from the patented Thylox liquid purification process. Ferrox is obtained in the patented iron-oxide liquid-purification process and after it is washed may contain from 2 to 6 per cent iron oxide in a very finely divided state. Gray sulphur is obtained in the liquid purification of water gas and is so called because of its gray color. These sulphurs do not differ from one another in so far as characteristics of the sulphur particle are concerned.

Preliminary experiments relative to the physical and fungicidal properties of one type of flotation sulphur were carried on by de Ong (2, 3) in California. From his results it seemed desirable to carry on further experiments along this line. Accordingly, an investigation of this sulphur was started under the administration of the Crop Protection Institute. The primary object was to carry on laboratory and field studies to determine the physical and fungicidal properties of flotation sulphur. The experiments here reported were those conducted in Illinois, where ample facilities were furnished by the Department of Horticulture of the Agricultural Experiment Station.

PHYSICAL PROPERTIES OF FLOTATION SULPHURS

Size of particles: A direct microscopic examination was made of the size of particles of flotation, ground wettable, ground dusting, and processed sulphur. Mounts of the wettable sulphurs were easily obtained by placing a small amount of sulphur in a test tube, adding water, and agitating vigorously. A few drops of the suspension were then drawn off with a pipette, placed on a clean glass slide, a cover slip was added, and microscopic examination made. The different dusting sulphurs were blown by means of a stream of compressed air onto glass slides. Examination of the dusting sulphurs was made without the addition of a cover slip. Figure 1 shows photomicrographs of wettable and dusting sulphurs. Table 1 contains particle-size measurements of a number of the sulphurs examined.

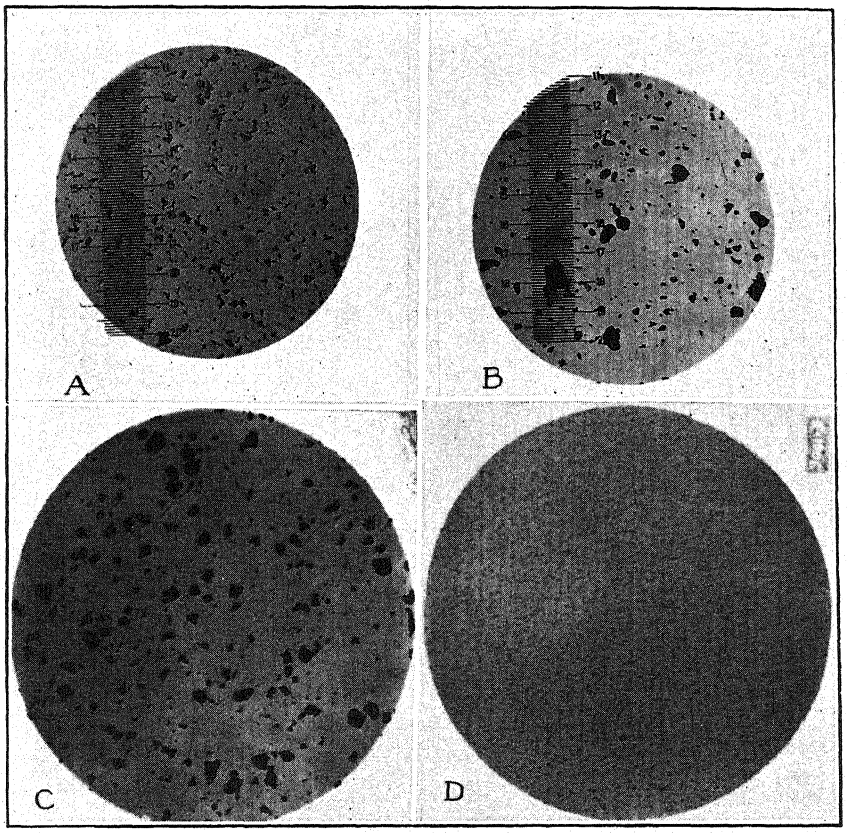


FIG. 1. (A) Flotation sulphur dust ×60; (B) Niagara 200 mesh dust ×60; (C) Niagara Super sulfo dust ×100; (D) Thylox flotation sulphur ×100. (Photomicrographs.)

TABLE 1.—*Comparative size of the particles of certain types of sulphur*

Sulphur	Smallest particles (microns dia.)	Largest particles (microns dia.)	Average size of particles (microns dia.)
Unwashed flotation sulphur[a]	1	5	3
Washed flotation[a] do	1	5	3
Ialine colloidal[a] do	1	3	2
Flowers of do	20	130	39
Mercks precipitated do	6	100	28
Ground roll do	8	200	46
Niagara 200 mesh dusting do	8	87	28
Flotation sulphur dust	3	5	4

[a] Many particles were less than one micron in diameter and too small for accurate measurement.

The results of these tests showed that the particles of Ialine and flotation sulphur were of the most uniform degree of fineness of any of those examined since approximately 95 per cent were 3 microns in diameter.

FOLIAGE INJURY TESTS WITH FLOTATION SULPHUR

The impurities in flotation sulphur as it leaves the filter press consist mainly of small percentages each of sodium thiosulphate and sodium thiocyanate. When each was tested on the foliage of bean plants at percentages ranging from 1 to 15 it caused injury at all concentrations. At concentrations of 5 per cent and above the injury was so severe as to cause complete desiccation of the foliage.

TABLE 2.—*Plant tolerance to unwashed and washed flotation sulphur*

Plant	Sulphur	Concentration	Injury
Bean	Unwashed	1–50	0
Do	do	2–50	Slight
Do	do	3–50	Severe
Peach	do	1–50	0
Do	do	2–50	Moderate
Cherry	do	3–50	Moderate
Apple	do	1–50	0
Do	do	2–50	Slight
Do	do	3–50	Severe
Gooseberry	do	1–50	Slight
Cineraria	do	2–50	Slight
Pelargonium	do	2–50	Severe
Ageratum	do	2–50	Severe
Primula malacoides	do	2–50	Slight
Bean	Washed	1–50	0
Do	do	2–50	0
Do	do	3–50	0
Do	do	4–50	0
Do	do	5–50	0
Do	do	6–50	Slight
Apple	do	2–50	0
Do	do	3–50	0
Do	do	4–50	0
Do	do	5–50	0
Do	do	6–50	0
Do	do	7–50	0
Do	do	8–50	Slight
Cherry	do	3–50	0
Do	do	6–50	0
Peach	do	2–50	0
Do	do	3–50	0
Do	do	4–50	0
Do	do	5–50	0
Rose	do	4–50	0
Do	do	5–50	0
Gooseberry	do	5–50	0
Verbena	do	4–50	0
Geranium	do	5–50	Slight
Cuphea	do	4–50	Slight
Primula kewensis	do	3–50	0

Tests were next made of the effect of unwashed and washed Thylox flotation sulphur on the foliage of a number of different plants. A list of the plants with the concentrations of unwashed and washed flotation sulphur which were tested on them appears in table 2.

The results of this experiment show that unwashed flotation caused injury to foliage in all but two instances when used at a concentration of 2 pounds to 50 gallons. It may be concluded that the impurities present in the unwashed sulphur were responsible for the injury to the foliage of the plants listed in table 2. Apparently the plant tolerance to washed flotation sulphur is much greater since in only three instances was injury observed at the higher concentrations.

DISEASE-CONTROL EXPERIMENTS

Spraying and dusting experiments at Ozark, Ill., in 1928: The use of sulphur dusts and sprays for the control of apple scab was tested at Ozark, Illinois, in 1928. The equipment consisted of a Bean power sprayer and a Bean power duster. In this orchard the trees were twenty-year-old Kinnairds and the plots consisted of 25 trees. The trees were so large that each required eight to ten gallons of spray material. A thousand apples were checked from each plot, the four center trees only being checked, and the apples were harvested in such manner as to obtain fruit from each quarter of the tree.

The results of the spraying experiment at Ozark, Illinois, in 1928, appear in table 3.

The results of the spraying experiments at Ozark show (a) that the standard spray, lime sulphur, was the only one giving satisfactory control of apple scab; (b) the wettable sulphurs, *i.e.,* Ialine, Koloform, Mulsoid, Thylox washed flotation, and Thylox unwashed flotation, at the concentrations used, gave unsatisfactory scab control; (c) injury to the foliage resulted from the use of unwashed flotation sulphur, thus confirming previous experiments which showed that injury resulted from the use of unwashed sulphur; (d) the fruit receiving washed flotation sulphur sprays had a superior finish.

The dusted plots at Ozark consisted of three plots of 36 trees each, so located that dust or spray materials from other plots would not be carried to the trees from which results were to be taken. The dusts were applied early in the morning at intervals corresponding to the spray applications. The three dusts used and the schedule followed were: Plot 16, home-mixed sulphur dust, following standard spray schedule, using 85–15 except in 4- and 6-weeks applications, at which times 100 per cent sulphur was used; Plot 17, Kolotex throughout season; Plot 18, Thylox flotation sulphur dust as in Plot 16—85–15 home-mixed.

TABLE 3.—*Efficiency of sulphur sprays for the control of apple scab. Ozark, Illinois. 1928*

Plot No.	Treatment	Percentage scab		
		Light	Severe	Total
1	Standard spray[a]	9.8	0.7	10.5
2	Unwashed Thylox flotation sulphur 1½–50 Schedule as in (1)	28.5	15.4	47.9
3	Lime sulphur 1–50 in prebloom; unwashed flotation sulphur 1½–50 in subsequent sprays	40.5	5.3	45.8
4	Washed Thylox flotation sulphur 2–50 Schedule as in (1)	36.2	20.2	56.4
5	Lime sulphur 1–50 in prebloom; washed flotation sulphur 2–50 in subsequent sprays	56.0	14.4	70.4
6	Washed Thylox flotation sulphur 3–50 Schedule as in (1)	45.1	7.1	52.2
7	Unwashed Thylox flotation sulphur 2–50 Schedule as in (1)	40.5	15.1	55.6
8	Ialine 1–50 Schedule as in (1)	27.7	10.3	38.0
9	Koloform sulphur 7½–50 Schedule as in (1)	34.0	36.8	70.8
10	Mulsoid sulphur 4–50 Schedule as in (1)	37.0	27.8	64.8
11	Commercial sprayed plot Schedule as in (1)	19.0	6.4	25.4
12	Check	2.0	98.0	100.0

[a] Prebloom (Apr. 10)
Calyx (May 3)
One week (May 14)
Two weeks (May 22)
Three weeks (May 31)
Four weeks (June 7)
} Lime sulphur 1–50 and lead arsenate 2–50

Six weeks (June 22)
Nine weeks (July 13)
3rd brood (July 28)
} Bordeaux 3–5–50

Lead arsenate 2–50 and hydrated lime 4–50 used in all applications.
Washed and unwashed flotation sulphurs used in this experiment on dry-sulphur basis.

Table 4 gives the results of the dusting experiment at Ozark, Illinois.

It is apparent from table 4 that none of the dusts was effective in controlling apple scab. Scab infection came late in the season and was very

TABLE 4.—*Effectivness of Sulphur dusts for the control of apple scab. Ozark, Illinois. 1928*

Plot No.	Treatment	Percentage scab		
		Light	Severe	Total
16	Home-mixed sulphur dust Following standard spray schedule, using 85–15 dust (300-mesh) except in 4- and 6-weeks, at which time 100 per cent sulphur dust was used	37.8	48.1	85.9
17	Kolotex—throughout season	16.4	70.1	86.5
18	Thylox flotation dusting sulphur Schedule as standard sprayed plot, 85–15 home-mixed	50.2	20.3	70.5
1	Standard spray plot, lime sulphur 1–50	9.8	0.7	10.5
19	Check	2.4	97.3	99.7

severe throughout this region. Weather conditions also were abnormal. There is a possibility that in a more normal year or in a year when scab infection is not so severe sulphur dust might prove more effective in the control of apple scab. However, these results tend to confirm those obtained by Anderson (1), namely, that dusting cannot be substituted for spraying for the control of apple scab in Illinois.

Spraying and dusting experiments in 1928 at Barry, Illinois: The spraying and dusting experiments at Barry, Illinois, were primarily for the control of apple scab. The orchard consisted of 26-year-old Ben Davis trees. The trees were large, requiring ten gallons of spray material to the tree. The equipment consisted of a Bean power sprayer and a Bean power duster. The sprayed plots contained 20 trees and the dusted plots 40 trees. Scab infection in 1928 was comparatively light, as is usual in this section of western Illinois. The crop was light and scattered due to late spring freezes which had killed most of the buds. A thousand apples from four center trees of each plot were used in determining the amount of scab present.

Table 5 contains the results of the spraying experiments at Barry, Illinois, in 1928. The results of these spraying experiments show (a) that the standard spray, lime sulphur, gave the best control of scab; (b) all wettable sulphurs gave fair control of scab; (c) injury to foliage resulted from the use of unwashed flotation sulphur; (d) in the plot receiving lead and lime, only, throughout the season, the incidence of scab was 44.7 per cent. These results confirm those obtained in previous years in Illinois, viz, that lead and lime are about 50 per cent as efficient for the control

of apple scab as is lime sulphur used at the standard strength. (e) Lime sulphur applied early, followed by Thylox flotation dusting sulphur applied late in the season (Plot 26), gave scab-free apples of excellent finish.

TABLE 5.—*Effectivness of sulphur sprays for the control of apple scab. Barry, Illinois. 1928*

Plot No.	Treatment	Percentage scab		
		Light	Severe	Total
1	Standard spray[a]	0.7	0	0.7
2	Unwashed Thylox flotation sulphur 1½–50. Schedule as in plot (1)	5.9	1.7	7.6
3	Mulsoid sulphur 4–50. Schedule as in plot (1)	6.5	2.7	9.2
4	Washed Thylox flotation sulphur 3–50. Schedule as in plot (1)	8.1	2.9	11.0
5	Koloform sulphur 7½–50. Schedule as in plot (1)	3.7	0.9	4.6
6	Washed Thylox flotation sulphur 4–50. Schedule as in plot (1)	9.3	4.5	13.8
7	Lead and lime Arsenate of lead 2–50 Hydrated lime 4–50	21.9	22.8	44.7
8	Washed Thylox flotation sulphur 6–50. Schedule as in plot (1)	4.0	0.7	4.7
9	Check	27.2	31.9	59.1
26	Lime sulphur 1–50 to nine weeks; flotation dusting sulphur at nine weeks; ten weeks, and three weeks before harvest	0.6	0.1	0.7

[a] Prebloom, Calyx, One week, Three weeks, Four weeks ..... Lime sulphur 1–50; lead arsenate 2–50.

Nine weeks—Bordeaux 2–5–50.

The dusted plots at Barry, Illinois, consisted of 5 plots of 40 trees each, one plot of 25 trees, and one of 15 trees. Dusting was generally done in early morning or in the evening. As in the experiment at Ozark, the percentage of scab was estimated from center trees within the plots. One thousand apples were checked from each plot.

The results in table 6 show that dusting gave only fair control of apple scab. Thylox flotation sulphur dust 85–15 and Thylox flotation sulphur-potassium permanganate dusts were about equally effective in the control of scab.

TABLE 6.—*Effectiveness of sulphur dust for the control of apple scab. Barry, Illinois. 1928*

Plot No.	Treatment	Percentage scab		
		Light	Severe	Total
20	Home-mixed sulphur dust, following standard spray schedule, using 85–15 dust (300 mesh). Pure sulphur alone in prebloom	4.2	2.8	7.0
21	Thylox flotation dusting sulphur 85–15, following schedule as in (20)	5.2	1.9	7.1
22	Thylox flotation dusting sulphur 85–15. Two prebloom applications, followed by 1-wk., 2-wk., 3-wk., 4-wk., 9-wk., 10-wk., and 3d brood applications	7.3	4.6	11.9
23	Thylox flotation dusting sulphur-potassium permanganate-lead arsenate 80–5–15. Schedule as in (20)	3.7	3.8	7.5
24	Kolotex—Schedule as in (20)	20.0	11.4	31.4
25	Kolotex—Schedule as in (22)	18.6	13.5	32.1
27	Check	11.8	8.8	20.6

That these results are somewhat inconsistent may be seen from the fact that flotation dusting sulphur applied seven times gave a total of 7.1 per cent of scab, while in the plot to which nine applications were made there was 11.9 per cent of scab. Furthermore, in both of these plots there was slightly poorer control of scab than in the home-mixed dust plot which had only seven applications of dust but yielded 7 per cent of scabby fruit. Observations made indicated that there was considerable variation in severity of scab in different portions of the orchard. It was noticeable that there was less scab infection at the west than at the east end. Both plots receiving Kolotex were at the east end of the dusted plots. The percentage of scab in these two plots was 31.4 and 32.2, respectively. Since both of these developed between 11 and 12 per cent more scab than the

check plot, it is quite evident that there was more scab in this portion of the orchard.

SPRAYING EXPERIMENTS AT BARRY, ILLINOIS, 1929

The purpose of the experiments at Barry, Illinois, in 1929, was threefold:

To test the fungicidal efficiency of flotation sulphurs; to supplement the later lime-sulphur applications with flotation sulphurs; and to observe the effect of the different sprays on the foliage of apple trees and on the finish of the fruit. The spraying was carried on in a block of 26-year-old Ben Davis apple trees, a portion of which had been used in 1928 in dusting and spraying experiments. The equipment consisted of a Bean power sprayer. About ten gallons of spray material was required for each tree.

Spray materials used were: Commercial lime sulphur, flotation dry mix—a material containing 70 per cent Thylox flotation sulphur and 30 per cent hydrated lime—Sulfuron, a commercial wettable sulphur, and flotation sulphurs of three types, all recovered in the process of gas purification. The use of unwashed flotation sulphur as a summer spray was discontinued in 1929 because of the injury produced from the impurities contained in it.

The spray plots each contained 20 trees. With two exceptions, each treatment was triplicated. Plots receiving similar treatments were located in widely scattered portions of the orchard. This precaution was taken to correct as nearly as possible any differences in scab severity prevalent in certain sections of the orchard. At harvest time a thousand apples were picked from different portions of four center trees of each plot. In estimating scab severity, apples having one conspicuous or two less conspicuous scab lesions were classed as "light scab." When more than this amount of scab was present, it was designated "severe scab." The results of the tests at Barry, Illinois, in 1929, appear in table 7.

Excellent control of scab was obtained in the standard sprayed plot. Moderate spray injury and slight defoliation were observed in the plots receiving this treatment. Good control of scab was obtained in the plots receiving flotation sulphurs. The finish of the fruit from these plots was excellent. No spray injury was observed. In Plots 8, 9, and 10, lime sulphur was used in early applications and washed Thylox flotation sulphur in subsequent sprays. As previously noted, such a schedule was planned for two reasons: (1) Lime sulphur to be used in early applications because of its high toxicity and at a time when scab infection is often most severe; (2) wettable sulphurs to be used in later sprays because of their ability to control scab appearing later in the season. In addition, the wettable sulphur sprays are much less likely to cause spray injury to the fruit and

TABLE 7.—*Effectiveness of sprays for the control of apple scab. Barry, Illinois. 1929*

Plot No.	Treatment	Average percentage scab		
		Light	Severe	Total
1	Thylox flotations dry mix 7½–50 in all sprays	5.93	2.10	8.03
2	Lime sulphur 1–50 in prebloom, flotation dry mix 7½–50 in subsequent sprays	8.47	4.53	13.0
3	Lime sulphur 1–50 in prebloom and calyx, flotation dry mix 7½–50 in subsequent sprays	6.63	3.50	10.1
4	Gray flotation sulphur 5–50 in all sprays	7.10	3.50	10.8
5	Thylox flotation sulphur 5–50 in all sprays	6.07	2.80	8.87
6	Ferrox flotation sulphur 5–50 in all sprays	2.63	0.30	2.93
7[a]	Standard spray, lime sulphur 1–50 in all sprays	3.40	0.11	3.51
8	Lime sulphur 1–50 in prebloom, Thylox flotation sulphur 5–50 in subsequent sprays	4.23	0.83	5.06
9	Lime sulphur 1–50 in prebloom (two applications), Thylox flotation sulphur 5–50 in subsequent sprays	1.62	4.10	5.72
10	Lime sulphur 1–50 in prebloom and calyx, Thylox flotation sulphur 5–50 in subsequent sprays	7.24	4.16	11.4
11	Check	10.83	84.1	94.9
12	Lead arsenate 2–50 and lime 4–50 in all sprays	16.70	35.9	52.6
13	Lime sulphur 1–50 and ferrous sulphate 3/4–50 in all sprays	3.30	0.56	3.86
16	Lime sulphur 1–50 in prebloom, Sulfuron 4–50 in subsequent sprays	15.1	13.0	28.1
A	Commercial-sprayed plot. Lime sulphur 1½–50 in all sprays	5.3	3.1	8.4

* Prebloom—(April 12)
Calyx—(April 23)
One week—(May 7)
Three weeks—(May 21)
Five weeks—(June 10)
} Lime sulphur 1–50; lead arsenate 2–50.
Nine weeks—(July 15) Bordeaux 2–5–50.
Lead arsenate 2–50 and lime 4–50 used in all sprays.
All flotation sulphur sprays used on dry-sulphur basis.

foliage, which often occurs when lime sulphur is used through most of the spray season.

Observations during the early part of the spray season showed that the period of maximum scab infection was about ten days later than ordinarily occurs in western Illinois and that the infection was most severe between the time of the calyx and the one-week sprays (April 29–May 4). The smaller percentages of scab in Plots 8 and 9 may have been due in part to the early applications of lime sulphur. On the other hand, the percentage of scab in Plot 10, receiving lime sulphur in the prebloom and calyx applications, was nearly double that in Plots 8 and 9. If lime sulphur was responsible for the decreased amount of scab in Plots 8 and 9, it apparently was ineffective in Plot 10. Inasmuch as the heaviest scab infection occurred after the lime-sulphur applications were made, no definite conclusions may be drawn from this test. However, it is believed that in years when scab appears early in the season the schedule of lime sulphur in early applications followed by wettable sulphurs should prove successful. In Plot 13, receiving lime sulphur and ferrous sulphate, the percentage of scab was about equal to that in the standard sprayed plot. No appreciable reduction in the efficiency of lime sulphur for scab control was observed through the addition of ferrous sulphate. There was, however, considerable



FIG. 2. Apple-spraying experiment. Unsprayed plot on left. Plot to right received flotation sulphur in all sprays.

spray injury from the use of this combination. This is contrary to the results obtained by Dutton and others (4), who report the successful use of iron sulphate and lime sulphur in combination as a preventive of spray injury. Defoliation in the check plots was very severe (Fig. 2). The influence of defoliation on the size of the fruit in this plot was a striking example of the necessity of holding foliage on the trees.

PEACH-LEAF-CURL CONTROL

A cooperative investigation between the State Natural History Survey and the Department of Horticulture at Urbana, Illinois, on the use of certain fungicides alone and in combination with oils for the control of peach-leaf curl and San Jose scale was undertaken in the fall of 1927.

The experiments were conducted in 18 plots in each of three orchards. Each plot contained 20 trees. Two check plots were located in orchard No. 1, two in orchard No. 2, and five in orchard No. 3. In estimating the amount of peach-leaf-curl infection, the following method was employed: An examination was made of at least one check plot in each orchard. The percentage of diseased old leaves was then estimated by examining each tree of the check plot, special attention being given to the inside trees near the center of the plot. New leaves were eliminated in these estimates, since none were diseased in any of the plots. An examination was next made of all the trees in each plot. An average was computed after an examination of all the trees.

Table 8 gives the record of the experiments carried on in the fall of 1927 and spring of 1928.

The results of peach-leaf-curl-control experiments as outlined in table 8 may be summarized as follows: Lime sulphur 1–8 gave very satisfactory control, whether applied in the fall or spring, and this season was effective when applied late in the spring. Oil emulsion with Bordeaux 4–6–50 gave satisfactory control in one orchard and rather poor control in the other two when applied in the fall. The curl control was somewhat less pronounced in two orchards in the spring application, but better in the third orchard. Oil emulsion with 4–50 copper sulphate gave very good control in all cases, in both fall and spring, as well as in late spring this season. It gave slightly more curl in the late-spring application than did lime sulphur. Unwashed Thylox flotation sulphur 7½–50, alone, gave complete leaf-curl control in both fall and spring applications.

Unwashed Thylox flotation sulphur 7½–50 and oil emulsion in a combination spray gave complete leaf-curl control in both fall and spring applications.

At Neoga, Illinois, a block of 900 five-year-old Elberta trees was used for the dormant spray tests. Two materials were used, dry lime sulphur,

TABLE 8.—*Effectiveness of different spray materials in the control of peach-leaf curl at Carbondale, Illinois, 1927–28*

Plot No.	Materials and strengths	Time applied	Percentage peach-leaf curl		
			Orchard No. 1	Orchard No. 2	Orchard No. 3
1	Lime sulphur 1–8	Fall	Less than 1	Less than 1	Less than 1
2	Emulso 3 per cent with lime sulphur 1–20	do	10	5	10–15
3	Scalecide 1–15	do	2	4–5	10–15
4	Oil emulsion 3 per cent with Bordeaux 4–6–50	do	Less than 1	Less than 1	10–15
5	do bluestone 4–50	do	Less than 1	Less than 1	2–3
6	do bluestone 2–50	do	3–4	4–5	5–8
7	do bluestone 4–50	do	3–4	Less than 1	Not applied
10	L–21[a] with unwashed flotation sulphur 15–50	do	No curl	Less than 1	Less than 1
11	L–21 with unwashed flotation sulphur 15–50	do	Not applied	Less than 1	1
13	Same as (1) but applied in	Spring	Less than 1	Less than 1	Less than 1
14	do (4) do	do	1–2	3	4–5
15	do (5) do	do	1–2	Less than 1	1
16	do (6) do	do	4–5	2	5
17	Scalecide, with Sulfocide 1–200	do	20–30	10–20	15–20
18	Same as (10)	do	No curl	Trace in one tree	1
19	Same as (11)	do	Less than 1	Less than 1	5
20	Same as (1) but applied in very late	do	No curl	Less than 1	Less than 1
21	do (5) do	do	No curl	1–2	3–4
Check			N. E. plot 30–50	N. W. plot 40–50	N. W. plot 40–50
do			N. W. plot 20–30	Center plot 10–15	S. W. plot 30–40
do					S. E. plot 30–40
do					E. plot 30–40
do					S. plot 20–30

[a] An experimental oil emulsion, not on the market in which Thylox was the flotation sulphur used.

12 pounds to 50 gallons, and unwashed Thylox flotation sulphur in combination with an oil emulsion prepared by the Standard Oil Company of Indiana. This combination was used at the rate of 7½ pounds of the oil-emulsion-sulphur mixture to 50 gallons. The spraying was done by the owner of the orchard, who used a power sprayer at a pressure slightly over 175 pounds. Spraying was not done until late in March, when the buds were just beginning to open on some of the trees.

The spray materials were applied to six-year-old Elberta trees. Dry lime sulphur was used in spraying eight rows containing 34 trees in each row. Thylox flotation sulphur-oil emulsion was similarly applied to an equal number of trees. Untreated rows were left at both ends and in the center of the block.

On May 17 final observations were made of the percentage of leaf curl in the sprayed and unsprayed rows. These observations showed: (a) 15–25 per cent of leaf curl in the rows receiving dry lime sulphur and oil-emulsion-sulphur sprays; (b) an average of 50 per cent leaf curl in the untreated rows; (c) no injury to the trees from either of these spray materials. From these results it is apparent that the sprays applied were only partially effective in reducing leaf curl. The lateness of application was very likely one of the main factors in the inability to obtain more efficient leaf-curl-control.

A similar test was made at Urbana, Illinois, in the winter of 1928. For this test four rows of seedling peaches were used. All materials (Table 9) were mixed in the field and applied with a Paragon sprayer.

On May 23, a final count was made of the number of curled leaves showing in this block of trees. Table 9 gives the results of the experiment.

TABLE 9.—*Effectiveness of certain sprays for the control of peach-leaf curl. Urbana, Illinois. 1928*

Row No.	No. trees	Treatment	No. curled leaves	Averaged number curled leaves per tree	Percentage leaf curl
1	17	Unwashed Thylox flotation sulphur and oil emulsion 7½–50	9	0.5	1.0
2	17	Dendrol 2 per cent and Bordeaux 4–4–50	20	1.2	2.5
3	18	Dendrol 2 per cent and Oxo-Bordeaux 4–50	180	10.0	20.7
4	12	Check	580	48.3	100.0

This experiment shows that the unwashed sulphur-oil-emulsion combination was highly effective in controlling peach-leaf curl. Though only slightly better than the Dendrol-Bordeaux spray, it was considerably more efficient than the Dendrol-Oxo-Bordeaux combination. No injury was observed from any of these treatments.

PEACH BROWN-ROT CONTROL

During the 1928 season a number of experiments were carried on to test (1) the effectiveness of dusting with sulphur for brown-rot control, (2) the relative merits of various commercial dusting sulphurs and home-mixed dusts, and (3) the relative merits of commercial wettable sulphurs, floatation sulphurs, and dry mix-sulphur-lime and self-boiled lime sulphur. These experiments were carried on in two orchards near Ozark, Illinois. Due to extremely light brown-rot infection throughout this section, no significant differences in brown-rot infection were observed in the treatments. As far as could be ascertained from detailed observations, none of the treatments had any detrimental effect on the foliage or fruit in any of the plots.

TABLE 10.—*Relative effectiveness of certain sprays for the control of brown rot of peach. Carbondale, Illinois, 1929*

Plot No.	Number trees	Treatment	Percentage brown rot
1	25	Dritomic 3–50	3.32
2	31	Sulfuron 3–50	1.88
3	34	New Jersey dry mix 12½–50	0.84
4	33	Self-boiled lime sulphur 8–8–50	1.29
5	28	Thylox flotation dry mix 3½–50	0.65
6	29	Thylox flotation sulphur 3½–50	0.61
7	35	Gray flotation sulphur 3½–50	0.51
8	25	Mulsoid 4–50	1.75
9	24	"Mist" brand 3½–50	3.04
10	25	Check	17.40
11	19	Koloform 7½–50	4.82
12	20	New Jersey dry mix 1/2 strength (6¼–50)	2.09

Dates of application of sprays:

April 16
do 22
May 10
do 15
June 4
July 2
do 17

In 1929, tests were made of the relative efficiency of various commercial wettable sulphurs, flotation sulphurs, and the standard home-mixed types of sprays, such as dry mix-sulphur-lime and self-boiled lime sulphur. All the commercial wettable sulphurs were used at the maximum recommended strength for peaches. The flotation sulphurs were used at the rate of $3\frac{1}{2}$ pounds to 50 gallons. Self-boiled lime sulphur was used at the recommended strength of 8–8–50. New Jersey dry mix was used at the recommended strength of $12\frac{1}{2}$ to 50 and also at the rate of $6\frac{1}{4}$ to 50. The spray materials were applied with a Bean power sprayer on the dates listed in table 10. As in previous experiments, the percentage of disease was obtained from fruit picked from center trees of each plot.

In table 10 it will be noted that the percentage of brown rot was small in all the plots receiving sulphur sprays. There were only slight differences among the wettable sulphurs. Apparently, the sprays applied to Plots 3, 5, 6, and 7 were the most effective in reducing brown-rot infection. There was no spray injury observed in any of the plots.

CHERRY-LEAF-SPOT CONTROL

During 1929, an experiment for the control of cherry-leaf spot was carried on in a nursery at Normal, Illinois. The experiment was planned to include the following spray materials: Bordeaux mixture 3–5–50, Ferrox flotation sulphur 6–50, and Gray flotation sulphur 6–50. Each of these materials was applied to two rows of 150 seedlings each of Montmorency cherries. Two rows of 150 trees each were left as checks.

The spray materials were applied with a sprayer of the bucket type. The first spray applications were made on May 29 when no leaf spot was observable. On June 20, at the time of the second application, some leaf spot was noted on a few trees in the check rows. On July 8, at the time of the third application, the disease was generally prevalent, many leaves on the trees in the nonsprayed rows had turned yellow and partial defoliation had occurred. Another application was made on August 9. Almost complete defoliation had occurred in most of the trees in the nonsprayed rows. In the sprayed rows there was no noticeable defoliation and almost no chlorosis had occurred.

On September 15, final observations were made of the sprayed and nonsprayed rows. The results appear in table 11.

From table 11 we may conclude that: (1) Bordeaux mixture 3–5–50 was the most effective of any of the sprays for the control of cherry leaf spot; (2) Ferrox flotation and Gray flotation sulphurs were about equal in fungicidal efficiency in this experiment.

Thylox flotation sulphur was used in comparison with lime sulphur for the control of cherry-leaf spot in the commercial cherry orchards at the

TABLE 11.—*Effectiveness of certain spray materials for the control of cherry leaf spot on seedling cherries. Normal, Illinois. 1929*

Row No.	Number trees	Treatment	Percentage defoliation
1	150	Ferrox flotation sulphur 6–50	4.0
2	150	do	5.0
3		Gray flotation sulphur 6–50	7.0
4	150	do	9.0
5	150	Check	75.0
6	150	do	85.0
7	150	Bordeaux 3–5–50	2.0
8	150	do	1.0

University fruit farm at Urbana, in 1929. This disease was successfully controlled with both of these materials until mid-season. No difference was observed between the two plots, both retaining a greater part of their foliage, while on nonsprayed trees severe defoliation had occurred at this time.

While these results represent only one season's work, they indicate the practical value of flotation sulphurs not only on nursery stock but on commercial cherry plantings. They may be applied when other sprays would injure the foliage or mar the fruit.

SUMMARY

1. A new type of sulphur, called flotation sulphur, obtained in the Koppers Liquid Purification Process of manufactured gas, was investigated. A preliminary report is given.

2. Particles of washed and unwashed flotation sulphur were of uniform fineness, ranging from 1 to 5 microns in diameter. In contrast, the particles of ground sulphur ranged from 6 to 200 microns in diameter.

3. Unwashed flotation sulphur produced injury to foliage because of the presence of the soluble impurities, sodium thiosulphate and sodium thiocyanate, which it contains.

4. In experiments conducted in the greenhouse, flotation sulphur washed free of impurities was safely used at concentrations higher than is ordinarily applied in fungicidal sprays. In field experiments in 1928 and 1929, no injury to foliage or fruit occurred when washed flotation sulphurs were used.

5. Apple-scab-control experiments with flotation sulphurs in southern Illinois in 1928 on a variety especially susceptible to scab were not successful. This may be ascribed in part to the low concentrations used.

6. In 1929 at Barry, Illinois, satisfactory control of apple scab was obtained with all types of flotation sulphur used.

7. No definite conclusions may be drawn from experiments as to the value of prebloom and calyx sprays of lime sulphur followed by flotation sulphur in remaining sprays. Failure to obtain more definite results on this point was probably due to the late appearance of apple scab in western Illinois in 1929.

8. The finish of the fruit from the plots receiving flotation sulphur sprays was good.

9. In the spraying experiment at Carbondale, Illinois, in 1929, the three types of flotation sulphur used gave somewhat better control of brown rot than did several brands of ground wettable sulphur.

10. Unwashed flotation sulphur, in combination with an experimental oil emulsion, satisfactorily controlled peach-leaf curl in experiments carried on in southern and central Illinois in 1927–28 and 1928–29.

11. Three types of flotation sulphur were used in cherry-leaf-spot-control experiments on nursery and commercial cherry plantings in 1929. Satisfactory control of the disease was obtained in these experiments.

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SOME SPECIES OF ATROPELLIS AND SCLERODERRIS ON CONIFERS IN THE PACIFIC NORTHWEST

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INTRODUCTION

Observations in the western part of Oregon, Washington, and British Columbia during the field seasons from 1923 to 1929 show that species of Atropellis (described as a new genus below) and Scleroderris are constantly associated with serious canker-like diseases of conifers. These observations have been made chiefly by workers in the Office of Forest Pathology and the Office of Blister Rust Control of the U. S. Department of Agriculture. With the advent of scouting for white pine blister rust, more particular attention has recently been called to the very deleterious effects of one of these affecting the western white pine, *Pinus monticola* D. Don., in many localities west of the Cascade Mountains and in Idaho. One example of this canker has recently been found on lodgepole pine,[1] *P. contorta* Loudon, and one on sugar pine, *P. lambertiana* Douglas.[2]. In Oregon a species of Scleroderris is believed to cause a canker of *Abies grandis* Lindl. and *A. amabilis* (Loudon) Forbes. For some time the fungus on the canker of western white pine and lodgepole pine has been referred tentatively by some mycologists to *Scleroderris bacillifera* (Karsten) Saccardo and to *S. treleasei* Saccardo by the writers. In order to throw some light on the taxonomy of these western species, the writers have made a comparative study of the fungi believed to cause these cankers of Abies and *P. monticola*, the type of *S. treleasei* which probably occurs on the bark of the Sitka spruce, *Picea sitchensis* (Bong.) Carrière, and a collection of apparently authentic *S. bacillifera*. The present paper discusses the economic importance of these diseases of *A. grandis*, *A. amabilis*, *Pinus contorta*, and *P. monticola* and also the comparative anatomy and taxonomy of the three species mentioned above.

[1] Common names of trees used in this paper are those used by Sudworth. Check list, Forest Trees of the U. S. U. S. D. A. Misc. Circ. 92. 1927.

[2] After this paper went to the editor, L. N. Goodding collected 75 cankers of *Atropellis pinicola* on sugar pine from just above Slate Creek in the Siskiyou Mountains, Josephine County, Oregon, February 17, 1930. On these cankers the pycnidia of *Fuckelia pinicola* were associated with *Atropellis pinicola*. The cankers have the same appearance as those described on *Pinus monticola*. The collection is Oregon Agricultural College Herb. No. 4886, Zeller Herb. No. 7611, and cankers have been sent to the U. S. Office of Forest Pathology, Portland, Oregon, and to Dr. J. S. Boyce, Yale University.

DISTRIBUTION AND ECONOMIC IMPORTANCE OF THESE FUNGI

So far as is known to the writers, *Scleroderris treleasei* has not been reported since the collection of the type in 1899. This was taken at Sitka, Alaska, by Dr. Wm. Trelease, and a collection by Prof. Trevor Kincaid taken at the same time was reported by Saccardo. There is a very small portion of the bark of the host with the type material in the Herbarium of the Missouri Botanical Garden. This is believed to be bark from the Sitka spruce, *Picea sitchensis*. No statement is made concerning its host relationship, whether parasitic or saprophytic.

The fungus on western white pine, *Pinus monticola*, has been found from Lane County, Oregon, north into British Columbia and eastward into Idaho. It perhaps occurs throughout the range of the host where moisture and temperature conditions are conducive to infection. The type locality perhaps should be designated as the Pacific Slope of British Columbia, Washington, and Oregon, although the type collection was taken near Quilcene, Jefferson County, Washington.

In locations where trees are infected the cankers may be only occasionally found or may kill many twigs and branches on small trees from 4 to 40 feet high. For the most part no infections are found on the main trunk of larger trees, but symmetrical growth of small trees is often prevented by cankers which kill out the main leader up where the diameter is small. The largest trunk cankers reported were about breast-high on trees 3 and 5 inches in diameter. Trees smaller than this often are killed by cankers girdling the trunks. Collectors scouting for white pine blister rust report that dead twigs and branches of *P. monticola*, killed by this fungus, closely resemble blister-rust "flags." In some localities in the Olympic National Forest, Washington, and the Mt. Hood National Forest, Oregon, very heavy infections are to be found. Individual trees up to 40 feet high may have many of their smaller branches and twigs killed by numerous cankers. On the smaller branches and on larger branches sunken cankers often occur on but one side of the branch and during the spring the bark on the last two years' growth of the cankers is usually spotted with numerous black apothecia of the fungus. Branches only partially girdled are nearly always thrifty but die when the girdle is complete.

The same fungus which occurs on *P. monticola* has been found but once on *P. contorta*. This one collection of 12 cankers from northern Coos County, Oregon, has given little opportunity to observe its economic importance on this host.

The Scleroderris on lowland white fir (*Abies grandis*) has been found in the Northwest in three locations, namely, at the head of Wilson River in Tillamook County, near Kingston in Linn County, and near Bayview, Lincoln County, Oregon. Most of the fir trees of this species are infected

in a limited area at the head of Wilson River. Further observations made since the discovery of the disease lead to the belief that the virulence of infection varies from year to year.

Twigs and branches, and trunks of trees up to 4 inches in diameter have been found affected with cankers of various sizes. There is no indication of growth of the canker after the initial year, so that the economic importance of its damage is not comparable to that caused by the perennial growth of the fungus attacking western white pine.

The same species of Scleroderris occurs on silver fir, *A. amabilis,* in Hood River County, Oregon. It has been reported from the one locality only. Apparently the damage is much less severe on this host than on *A. grandis.*

INFECTION AND PATHOGENECITY OF THE FUNGUS ON PINUS MONTICOLA

It is not known just when infection takes place, but some collections taken in May show some very small cankers, 6 to 7 mm. in diameter. These appear like late infections after the host tissues have begun to grow. The growing tissues of the host have excluded the further advance of the fungus by a cork layer before the infection reached the cambium. This would lead to the belief that for normal development of typical cankers the infection would take place in the fall or early winter. Another observation leading to the belief that infections take place in the fall is the fact that the excipular enclosures of the apothecia rupture when moistened artificially. Apothecia on the two-year-old ring of canker development are mature and open, many of them having discharged their spores. Those on the last year's canker development are mature but not open. These were collected from March to August. Since these latter apothecia are mature and readily rupture and expand upon the application of water it would seeem that ascospore liberation would take place with the first continued rains in the fall and the natural supposition would be that infection would follow during the dormant period of the bark and cambium of the host.

No attempts have been made to induce infection and canker formation by artificial inoculation. Our observations on pathogenicity of this organism causing the canker of *P. monticola* were made wholly from cankers induced under natural conditions of infection. It is not difficult to deduce from such observations, however, that this species is quite parasitic in nature. Most infections are apparently through uninjured bark or leaf-scars. Crotch infections are rather numerous. In many of these the smaller lateral branches are dead. It is believed that most of this "flagging" is caused by the canker. In fact, there is no indication that infection ever takes place through dead twigs. On the contrary, there is considerable evidence that the fungus does not grow saprophytically in the dead portions

of branches killed by girdling cankers. In cases where cankers have caused such dead flags one never finds apothecia beyond the limits of the last year's advance of the canker.

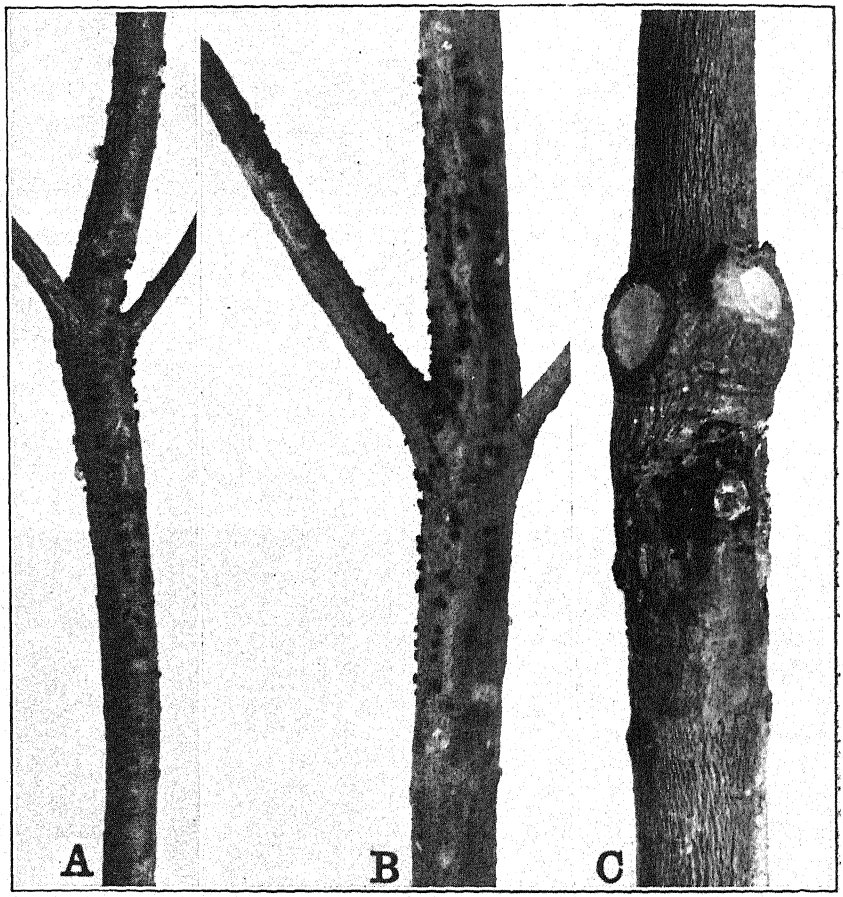


FIG. 1. Cankers on *Pinus monticola* with which *Atropellis pinicola* is associated. A. Three-year-old canker. The black bodies are apothecia. This canker was collected in November and shows no apothecia on the youngest or outer ring of dead wood. B. Three-year-old canker. Note the concentric rows of apothecia on the different ages of cankered bark. There are some pycnidia in the outer row of fruiting bodies. Collected in August. C. *Fuckelia*-stage collected in June, 1923, by H. G. Barstow at Chico, Kitsap County, Washington. Slightly reduced.

DESCRIPTION OF CANKERS ON PINUS MONTICOLA

The typical branch canker on *Pinus monticola* is caused by the killing of the cortex in long strips extending lengthwise of the limb (Fig. 1, A and

B). The affected tissues of the canker becomes somewhat sunken, but there is not an extreme shriveling nor separation from the surrounding healthy bark. Thus, for a number of years, the canker remains closed. The disease is progressive or perennial and year after year the canker encroaches upon the surrounding healthy bark. The greatest canker enlargement, for the most part, is lengthwise of the stem rather than around it. For instance, a canker 10 cm. long on a branch 1.5 cm. in diameter will be about $\frac{1}{4}$ as broad and may extend 3 cm. at each end during the second year, while advancing merely 6 to 10 mm. laterally during the same period.

Generally, the cankers are rather smooth, giving a flattened appearance to the side of the branches harboring them. On three-year-old cankers the central or oldest portion of the canker is usually dotted with disintegrating apothecia of *Atropellis pinicola*. The second year of the canker is marked by a line of mature, open apothecia arranged concentrically around the older portion, while on the last year's growth or youngest portion of the canker are to be found apothecia with mature asci but in which the excipulae have not yet ruptured above the hymenia. This is particularly true in cankers observed before the first rains in the fall of the year. Often on this last year's growth are to be found the black stromata of what we believe to be the imperfect stage. We have not demonstrated the organic connection between this imperfect stage and the ascogenous stage but their constant association on cankers gives circumstantial evidence. The only evidence of the causal organism on some one-year-old cankers is this pycnidial form (Fig. 1, C).

The cankers on *P. contorta* are very similar in appearance to those on *P. monticola*.

Cross-sections of cankers show rather deep staining of the wood. This stain is bluish black, reminding one of the stain produced by *Cenangium piniphilum* Weir. It occurs in the wood of both *P. monticola* and *P. contorta*.

INFECTION AND PATHOGENICITY OF THE SCLERODERRIS ON ABIES

As is true of the fungus attacking western white pine, the organism attacking *Abies grandis* and *A. amabilis* apparently should be considered a true parasite. In most of the cankers examined, if not all of them, infection took place through uninjured bark or leaf scars, so far as can be determined. The same arguments given to indicate that the infection on western white pine takes place in the fall of the year after continuous rains have begun hold also for infection by the fungus causing canker on fir. There are no indications of the fungus growing saprophytically under natural conditions.

DESCRIPTION OF CANKERS ON ABIES

In the main it would seem that this canker develops much more rapidly on *Abies grandis* than the one on western white pine. The development of the canker on *A. amabilis,* however, seems to be much slower than that on *A. grandis.* The spots develop continuously after once started during the dormant period of the host.

On *A. grandis,* the cankers are very smooth, there being no appearance of concentric zonation due to alternatng periods of rapid and slow growth (Fig. 2, A, B, and C). In early spring when canker enlargement ceases, the bark of the diseased areas dries and sinks, and often cracks longitudinally due to radial and tangential shrinkage. This condition, together with the normal growth of callus in the surrounding bark and wood, produces a tension which results in the formation of a crack in the bark at the

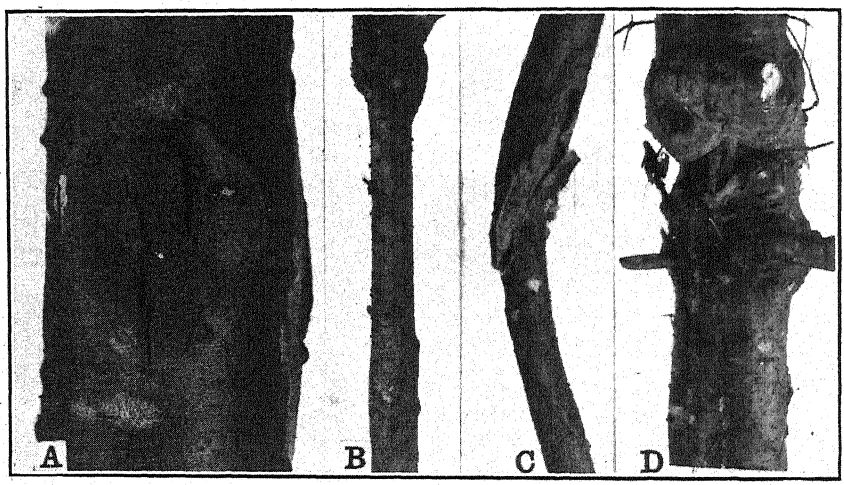


FIG. 2. Cankers on *Abies grandis* with which *Scleroderris abieticola* is associated. A. Young canker collected in October shows mature apothecia. Natural size. B and C. Small branches constricted by cankers become enlarged above when girdling it not complete or when the wood cylinder remains alive. Natural size. D. Old canker on a 4-inch trunk. ¼ diameter.

edge of the canker (Fig. 2, C). It is quite evident that the spread of the fungus ceases as soon as the cambium becomes active in the spring. These young cankers have a reddish brown color. Young cankers, collected May 24, 1928, show no apothecia, while young cankers collected October 18 show apothecia well developed but not yet dehiscent. This leads to the assumption that apothecia are developed during the summer of the first year of the canker. Observations so far show that the canker does not

develop farther than the first year's growth. The dead bark usually falls away during or after the second year and, if the branch or bole is not girdled the first year, the wound becomes completely calloused over.

On *A. amabilis* the cankers on young branches are very similar to those on *A. grandis,* showing the same type of constriction. The limited material available does not show the callus formation and cracking along the margins of cankers as illustrated by much of the material on *A. grandis.* One canker from a larger branch shows callus formation and marginal cracking and also indicates that the cankers do not increase in size after the first year.

TAXONOMY

After a study of the morphology of the fungi involved in the diseases discussed in this paper, we present herewith the following diagnoses. The first conception of the writers in their study of the three fungi involved was to refer them to Scleroderris Fries. There is apparently no question that the species on Abies should be so referred. We believe this to be distinct from other described species and therefore propose for it the name *S. abieticola,* sp. nov. Saccardo's *S. treleasei* and the fungus causing canker of *Pinus monticola* and *P. contorta* differ from the usual conception of the genus Scleroderris in two well-defined characters, *i. e.,* nonseptate spores and the color of the paraphyses and hymenium. We therefore refer these two species to a new genus, Atropellis. Saccardo's[3] statement relative to *S. treleasei* shows that he had leanings in this direction. He placed the species in the Dermateaceae but appended the following note (translated from the Latin): "Its relationships are a little doubtful, tending in some respects toward *Clithris,* in others toward *Coccophacidium* and in still others strongly toward a new generic type." In the Sylloge Fungorum he later added the clause "connecting closely with the Scolecosporae near Godronia."

ATROPELLIS Zeller & Goodding, gen. nov.[4]

Apothecia erumpent, single or caespitose from an inconspicuous stroma, sessile or stipitate, cupulate to broadly discoid, closed, at length laciniately-dehiscent, externally smooth to roughened, dark; *hymenium* variously colored; *asci* cylindrical to slightly clavate, 8-spored; *spores* filiform to acicu-

[3] Harriman Alaska Expedition, 5: Crypt. Bot. 1904.

[4] *Apothecia* erumpentia, singulares vel caespitosae, e stromatibus inconspicuibus, sessiles vel stipitatae, cupulatae ad disciformes; primere clausae, ultimate dehiscentesque laciniatae; exterior apothecii ater, levis vel asper; *hymenium* varie coloratum; *asci* cylindracei aut clavati anguste, octospori; *sporidia* filiformia vel aciculares, continua, hyalinia; *paraphyses* filiformes, simplices vel furcatae; ascos excendentis tenuiter aut extendatae et epithecium formatis, coloratae clare et varie; excipulum carbonacium; hypothecium crassum.

lar, continuous, hyaline; *paraphyses* filiform, simple or furcate, slightly exceeding the asci or extended and forming an epithecium, distinctly and variously colored; excipulum carbonaceous; hypothecium well developed.

The type species is *Atropellis pinicola* Zeller & Goodding.

Because of the thick, well-developed hypothecium or medullary tissues of the apothecia, the genus Atropellis belongs in the family, Tryblidiaceae. With Scleroderris it forms a scolecosporous group within the family and is related to other families of the Discomycetes through genera having needle-shape spores.

1. **Atropellis treleasei** (Sacc.) Zeller & Goodding comb. nov. *Scleroderris treleasei* Saccardo, Harriman Alaska Expedition, Vol. 5, Crypt. Bot., p. 24. *Pl. 3, fig. 7a–g.* 1904.

Apothecia solitary, or gregarious, at first erumpent, then entirely superficial, mostly sessible, at first pitcher-shaped, closed, then scutellate, laciniately-dehiscent, 2.5–4 mm. in diameter, expanding to 3–5 mm. when moistened; *outside* and *margins* torn, dusky purplish gray (Ridgway), carbonaceous, rugose; *hymenium* flatly-concave to convexly expanded, waxy, "pinkish cinnamon to Sayal brown" (Ridgway); *asci* clavate with obtusely-acute apices, narrowly and long stipitate, 100–178 x 8–14 μ (average 100 μ without stipe, 167 μ with stipe), eight-spored, staining brown with iodine; *paraphyses* filiform, with simple or incurved, furcate tips which very slightly exceed tips of asci, hyaline; *epithecium* none; *spores* fasciculate in upper part of ascus, mostly filiform, often somewhat clavate, hyaline, continuous, 42–60 x 2–2.5 μ; *hypothecium* a thin layer of light brownish prosenchyma underlaid by a slightly thicker layer of thin-walled, hyaline pseudoparenchyma which becomes less distinct toward the margin of the disc. The *excipulum* is composed of pseudoparenchyma in 3 layers in most specimens. The ectal layer is thin, dark brown (almost black), of small, polygonal cells with heavy walls. The layer just inside of this is of lighter brown cells of the same shape but a little larger. In some specimens the medullary hyaline tissue is a meshy pseudoparenchyma, as shown in plate I, B), but in others the type of pseudoparenchyma just above it extends to the second ectal layer just below. The *stem* is of the same structure as the excipulum. The stem arises several cells deep in the cortex of the bark.

On bark of *Picea sitchensis* (?); Alaska. The type was collected June, 1899, and, so far as the writers are aware, no other collections have been reported.

Specimens examined: Alaska: Baranof Island, Sitka, June, 1899, *William Trelease,* 845, type of *Scleroderris treleasei* (in Missouri Botanical Garden Herb.).

Godronia splendida Speg. must be closely related to *A. treleasei* and perhaps should be referred to Atropellis. It is described as having a beautifully golden hymenium and continuous spores.

2. Atropellis pinicola Zeller & Goodding, sp. nov.

Apothecia solitary or gregarious, erumpent from outer cortical layers of bark, sessile or on very short central stalk, 2–4 mm. in diameter, at first closed, opening by stellate or irregular clefts, leaving rather fimbriate margins, expanding discoid, usually rolling up from two sides when drying, externally pruinose, black to fuscous-black; *hymenium* pruinose, black; *asci* clavate, hyaline, staining brown with iodine, 8-spored, 74–178 x 8–13 μ (average 100 x 11 μ); *spores* filiform to acicular-clavate, hyaline, continuous, guttulate, 32–63 x 1.5–3.5 μ, (average 40 x 2 μ); *paraphyses* hyaline, hair-like, flexuous, exceeding the length of the asci by 32–38 μ, tips slender, agglutinated, forming a dense *epithecium* with rosy and purplish tints in section; *hypothecium* well developed, of dark brown, fascicled hyphae, extending mostly centrifugally upward from a hyaline, prosenchymatous, medullary portion of the apothecium; *excipulum* a prosenchyma, the ectal portion of which is composed of coarse, very dark-walled hyphae, which gradually shade lighter toward the hyaline medullar tissue.

Imperfect stage usually associated with *A. pinicola:*

Fuckelia: Stromata erumpent, sometimes scattered, mostly gregarious, black, pulvinulate, sessile to short-stipitate, 0.8–1.2 mm. in diameter, containing 16–35 locules (pycnidia); *stromatic tissue* of pseudoparenchyma surrounded by a carbonous rind of the same structure; *stipe* of same structure; *pycnidia* ovoid to globose, somewhat crowded, mostly in one plane, walls prosenchymatous, lining the stromatic pseudoparenchyma, 60–150 μ broad, 200–260 μ deep, ostiolate; *ostiole* slightly papillate, 25–32 μ in diameter; *conidiophores* from entire inner surface of pycnidia, hair-like, simple, and branched; *conidia,* hyaline, continuous, narrowly ellipsoid to bacillar, 8–11 x 1.7–3 μ.

To our knowledge this is the first report of a Fuckelia on conifers. There were previously three species described, two of which are designated as imperfect stages of species of Cenangium. None of the three, however, agrees in description with this one. Should this prove to be organically distinct from *Atropellis pinicola* it should be designated as *Fuckelia pinicola* Zeller & Goodding.

The pruinose character of the exterior of the apothecium is due to ectal hyphae projecting somewhat from the surface in tufts. In young closed specimens the excipulum is continuous over the top enclosing a collapsed mass of dark hyphae above the epithecium. This mass disappears when the excipulum ruptures above.

Associated with canker on living branches and trunks of *Pinus monticola* and *P. contorta.* Collections with the fungus in fruiting condition have been taken throughout the year. Oregon, Washington, Idaho, and British Columbia.

Specimens examined:

Montana: Flathead County, Olney, July 25, 1927, *C. R. Stillinger,* 2608 (in Zeller Herb., 7481).

Idaho: Clearwater County, Elk River, July 12, 1924, Sept. 1, 1928, and Sept., 1924, *C. R. Stillinger,* 593, 2605, 2609 (in Zeller Herb., 7476, 7475, 7474); Jericho, July 5, 1925, *C. R. Stillinger,* 544 (in Zeller Herb., 7477).

Oregon: Clackamas County, Government Camp, Jan. 1, 1927, *J. S. Boyce,* 1730 (in Zeller Herb., 7538, For. Path Herb.,[5] 40339); Still Creek, near Government Camp, July, 1927, *L. N. Goodding* (in Ore. Agr. Coll. Herb., 4931, and Zeller Herb., 7095); Rhododendron, May 30, 1924, *J. S. Boyce,* 1304 (For. Path. Herb., 40338); July 9 and 10, 1929, *G. D. Darker,* and *L. N. Goodding* (in Ore. Agr. Coll. Herb., 4874, 4875, and Zeller Herb., 7574, 7575); June 9, 1927, *L. N. Goodding* and *R. L. McLeod,* For. Path. Herb., 40390 (in Zeller Herb., 7163, and Ore. Agr. Coll. Herb., 4921); Zig Zag, Mt. Hood, July 11, 1926, *H. G. Lachmund* (For. Path. Herb.); Zig Zag Ranger Station, April 6 and May 15, 1929, *L. N. Goodding* (in Ore. Agr. Coll. Herb., 4876, and Zeller Herb., 7576 and 7578); July 11 and 15, 1929, *G. D. Darker* and *L. N. Goodding,* Yokum Falls (in Ore. Agr. Coll. Herb., 4877, and Zeller Herb., 7577, 7573); Coos County, ten miles south of Reedsport, Nov. 24, 1929, *L. N. Goodding* (in Ore. Agr. Coll. Herb., 4882, in Zeller Herb., 7589); Lane County, near Summit, McKenzie Pass, July 29, 1928, *L. N. Goodding* (in Ore. Agr. Coll. Herb., 4930, and Zeller Herb., 7457); Linn County, Pine Ridge, Big Meadows, July 16, 1926, *F. P. Sipe,* 40 (in Ore. Agr. Coll. Herb., 4878, and Zeller Herb., 7023); Wasco County, Barlow Creek Canyon, Aug. 10, 1928, *E. L. Evinger* (in Ore. Agr. Coll. Herb., 4929, and Zeller Herb., 7458).

Washington: Clallam County, Lake Crescent, Aug. 11, 1926, *J. S. Boyce* 1679 (For. Path. Herb., 40375); Jefferson County, Irondale, May 5, 1926, *L. N. Goodding* and *F. A. Patty* (in Ore. Agr. Coll. Herb., 4928, and Zeller Herb., 7086); Quilcene, Mar. 7, 1926, *S. B. Detwiler* and *L. N. Goodding,* type (in Ore. Agr. Coll. Herb., 4927, Boyce Herb., 1635, and Zeller Herb., 6892); Kitsap County, Chico, June 7, 1923, *H. G. Barstow* (in For. Path. Herb., 40389); Mason County, Sept. 3, 1927, *C. R. Stillinger,* 2606 (in Zeller Herb., 7473); Pierce County, Ashford, Aug. 16, 1927, *C. R. Stillinger,* 2607 (in Zeller Herb., 7471); near entrance to Rainier National Park, Aug. 15, 1927, *C. R. Stillinger,* 2610 (in Zeller Herb., 7472); Skamania County,

[5] In each of these cases, reference is made to the accession number in the herbarium of the Office of Forest Pathology, Post Office Building, Portland, Oregon.

above Wind River Forest Nursery, June 19, 1928, *L. N. Godding* (in Ore. Agr. Coll. Herb., 4926, and Zeller Herb., 7459); Nov. 28, 1927, *C. C. Strong* (in Ore. Agr. Coll. Herb., 4925, and Zeller Herb., 7460); Willard, June 12, 1924, *J. S. Boyce,* 1305 (in For. Path. Herb., 40340).

Canada: British Columbia, upper Birkenhead River Crossing, Cabin on Prohibition Claim Trail, Mile 72, P. G. E., May 25, 1927, *C. N. Partington* (in For. Path. Herb., 40426); upper Birkenhead River Valley, on Birkenhead Tenquil Trail, Mile 72, P. G. E., Sept. 1, 1927, *C. N. Partington* (in For. Path. Herb., 40427).

SCLERODERRIS FRIES

1. *Scleroderris abieticola* Zeller & Goodding, sp. nov.

Apothecia single or gregarious, from an erumpent stroma (or single apothecia erumpent from outer cortical layers of bark), on short central stalk, seldom sessile, 0.5–1.2 mm. in diameter, at first closed and spheroidal or ellipsoidal, opening by stellate or irregular clefts, then cupulate to expanded, externally smooth to flaky, grayish black to shiny black; *hymenium* shiny slate color to shiny black; *asci* clavate, hyaline, stained brown with iodine, but bore at tip blue, usually 8-spored, rarely 4-spored, 118–135 x 9–14 μ (average 129.5 x 11 μ); *spores* filiform, many guttulate, distinctly 5–8-septate, hyaline, 40–67 x 3.5–4.5 μ; *paraphyses* hyaline, hair-like, tips slightly enlarged, slightly exceeding the length of the asci, somewhat agglutinated but not forming an appreciable epithecium; *hypothecium* hyaline, composed of a thick, very gelatinous prosenchyma; *excipulum* with a black rind, underlaid by several layers of differentiated pseudoparenchyma. The excipulum extends downward forming the outer layers of the stem. The medullary region of the stem is pseudoparenchyma composed of brown, thick-walled isodiametric cells. From these there is a gradual transition through thin-walled, hyaline, pseudoparenchyma to the hypothecium.

The excipulum exhibits three rather well-differentiated layers with an external rind. The ental layer is pseudoparenchymatous, composed of small, dark, thin-walled, isodiametric cells. The middle layer joins the inner one with a gradual transition to cells appearing like rather small, parallel, light-brown hyphae coalescing to form a typical pseudoparenchyma. The ectal layer resembles the middle layer but the cells are distinctly larger, more barrel-shaped, with light amber-colored walls. The rind seems to be merely a darkened layer of the latter which may flake off, giving the grayish black appearance to some apothecia.

A very interesting phase of abnormal spore formation is sometimes to be observed in this species. Four-spored asci are not infrequently found. In fact, on first observation, the writers believed the species to be characterized by 4-spored asci. In all cases of subsequent examination, how-

ever, we have found the 8-spored asci to greatly predominate. The spores from both the 8-spored and 4-spored asci are distinctly septate. In some cases there are four large spores and four very small spores. These two types of abnormal asci are shown in Plate I, Q. They are usually somewhat shorter than normal 8-spored asci. Spores from 4-spored asci are 85–100 x 4.5–6 μ, while in asci containing four large and four small spores the larger are 70–80 x 4.5–6 μ and the small spores are 11–15 x 4–6 μ.

The writers first believed this Scleroderris, as it occurs on *Abies grandis*, was the same as the European *S. bacillifera,* but it has since been our privilege to study a collection of the latter on *A. pectinata* DC. This Eureopean material was collected in Bavaria, June, 1881, by Britzelmayr and is reported as *Tympanis bacillifera* Karsten on *Picea excelsa* L. This agrees in every way with Karsten's description. *Scleroderris abieticola* differs from this material and the description of the color and structure of the hypothecium, size of asci and spores, and general appearance. If the hyaline, gelatinous layer which we have referred to as the hypothecium could be considered as a medullary portion of the excipulum, the species should perhaps be referred to Godronia.

This species of Scleroderris is associated with cankers on living branches and twigs of *A. grandis* and *A. amabilis.* Collected in January, May, and October. Oregon.

Specimens examined:

Oregon: Hood River County, near Horse Thief Meadows on Mt. Hood Loop Highway, May 20 and July 6, 1929, *L. N. Goodding* (in Ore. Agr. Coll. Herb., 4873, 4920, and in Zeller Herb., 7247 and 7572); Lincoln County, near Bayview, Aug. 30, 1929, *S. M. Zeller* (in Zeller Herb., 7539); Linn County, Kingston, Jan. 6, 1928, *J. L. Mielke* (in For. Path. Herb., 40474, and Zeller Herb., 7470); Tillamook County, Head of Wilson River, May 24 (2 collections), Oct. 18, 1928, and Sept. 16, 1929, *L. N. Goodding* (in Ore. Agr. Coll. Herb., 4932, 4933 type, 4934, 4924, and in Zeller Herb., 7442, 7443 type, 7451 and 7541).

Another species of Scleroderris on *A. amabilis* was collected by G. D. Darker and L. N. Goodding near Horse Thief Meadows, Hood River County, Oregon, July 6, 1929. The material is so meager that we hesitate to refer it definitely to a species at this time. It apparently has close affinities with *S. amphibola* (Mass.) Gill. but differs from the latter in some characters. It seems to be entirely saprophytic.

SUMMARY

This paper describes as new a genus of fungi near Scleroderris in the family Tryblidiaceae. The name Atropellis is proposed for the genus with *A. pinicola* Zeller & Goodding, sp. nov., as the type species. A canker of

Pinus monticola and *P. contorta* with which *A. pinicola* is associated, and a canker of *Abies grandis* and *A. amabilis* with which *Scleroderris abieticola* Zeller & Goodding, sp. nov., is associated, are described and the distribution and economic importance of the two cankers are given. The paper includes diagnostic descriptions of the new genus Atropellis and the three fungi *Atropellis pinicola, A. treleasei,* and *S. abieticola.*

EXPLANATION OF PLATE I

Illustrating the morphology of *Atropellis treleasei, A. pinicola,* and *Scleroderris abieticola.* All drawings sketched to scale by means of camera lucida.

Atropellis treleasei

A. Diagrammatic vertical section of an apothecium, showing relation of parts and relation to host. ×10.

B. Vertical section through the margin of an apothecium, illustrating the structure of the excipulum, medullary tissue, hypothecium and hymenium, ×75.

C. Mature asci and paraphyses. Note the long stipitate asci with fascicle of spores in upper half and note that the length of paraphyses does not exceed that of asci. ×500.

D. Ascospores. ×500.

E. Shows the relation of the spores to the apex of an ascus. ×1500.

Atropellis pinicola

F and G. Two diagrammatic vertical sections of mature apothecia showing relation to host bark and relation of parts. Note in the closed apothecium that the space above the epithecium and within the excipulum is stuffed with a mass of collapsed hyphae. ×10.

H. Vertical section through the margin of an apothecium, showing grossly the morphology of the parts. Note the well-developed *hypothecium* and *epithecium.* ×75.

J. Asci and paraphyses. Note how the latter greatly exceed the length of the asci. ×500.

K. Ascospores. ×500.

L. Diagram of a vertical section of a stipitate stroma of the Fuckelia stage. ×10.

M. Vertical section through half of a pycnidial stroma showing the gross morphology of the parts. ×75.

Scleroderris abieticola

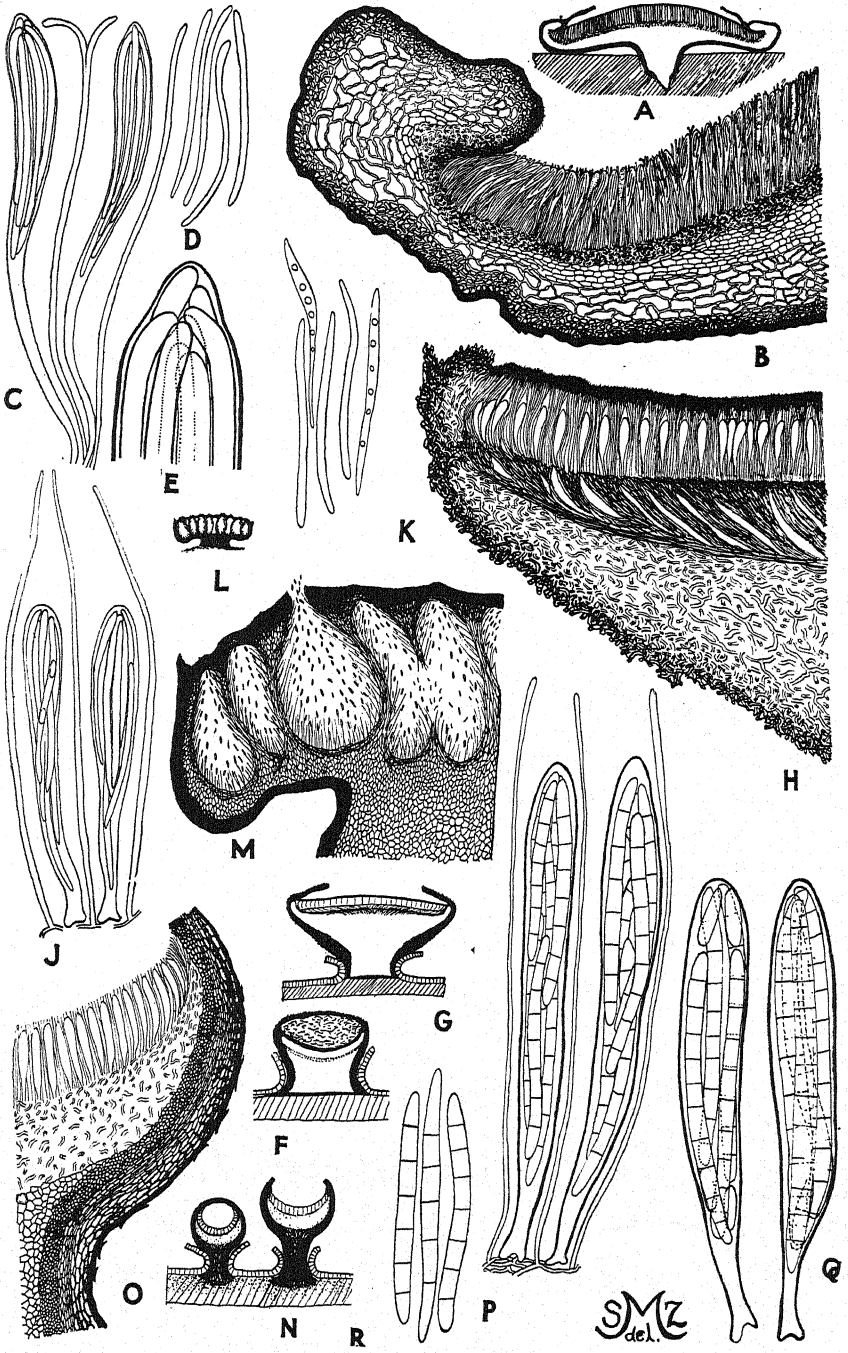
N. Diagram of vertical sections of both a closed and an open mature apothecium. The closed apothecia are almost spherical and more stipitate than in the other two species described here. ×10.

O. Vertical section through half of an apothecium, showing the gross morphology of the parts. Note particularly the complex structure of the excipulum and stipe. The hypothecium is merely a hyaline, gelatinous medullary region of the apothecium. ×75.

P. Asci and paraphyses. The length of the latter does not greatly exceed that of the asci. ×500.

Q. Two types of abnormal asci frequently seen. In one type there are 4 abnormally large ascospores; in the other are 4 large spores and 4 very small or extremely aberrant spores. ×500.

R. Ascospores. ×500.


A
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APPARATUS AND METHOD FOR OBTAINING STERILE FILTRATES OF BIOLOGICAL FLUIDS

EMERY R. RANKER[1]

INTRODUCTION

During a study of the physiology of disease resistance certain investigations were undertaken that involved obtaining sterile nonheated filtrates of the expressed juices of various parts of the host material involved. These filtrates were to be used in a comparative study of the effect of filtrates from resistant hosts and from susceptible hosts on the growth of the pathogene when cultured in those filtrates.

The number of hosts to be tested, the quantity of host material to be used, and the hundreds of sterile filtrates to be obtained made it imperative that any filtration apparatus used should meet certain definite requirements. These were: (1) The apparatus should be capable of producing absolutely sterile filtrates consistently when properly handled. (2) Once set up, it should not require further attention during the process of filtration until disconnected to obtain the sterile filtrate. (3) The apparatus should be constructed of parts that could be made in any laboratory by any one having some skill in glass blowing. (4) It should be compact, easy to disconnect to obtain the filtrates and to assemble for sterilization. (5) The receptacle for collecting the sterile filtrate should be constructed to facilitate subsequent transfer of the filtrate to various culture vessels without undue danger of contamination. (6) The apparatus should be so constructed as to permit sterilization in an autoclave after it is completely assembled, namely, so that but two operations are required to start the filtration process, (a) to connect to a source of vacuum and (b) to cover the surface of the filter with the juice to be filtered.

It is obvious that this sixth requirement and the large number of filtrates to be obtained would prohibit the use of agar, paraffin applied hot, and similar materials that frequently are recommended as suitable for sealing joints. Such substances, when so used, are unsatisfactory and it is advisable, when possible, to avoid them. The repeated use of heated paraffin to seal a joint adjacent to a porous filter surface ultimately renders that surface useless as a medium for filtration. This is true especially if the filter is alternately sterilized, even though the paraffin may have been carefully removed.

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A review of the literature revealed a dearth of described apparatus that could be used satisfactorily for this work. The apparatus used and described by McBryde[2] seemed to meet certain requirements, but the suction flask is rather complicated in construction and the calibrations are not sufficiently accurate. This apparatus makes use of the Chamberland-Pasteur filter. When uniformly sterile filtrates are desired, this has a decided advantage, as pointed out by McBryde, over the standard equipment consisting of a glass mantle attached to a Berkefeld filter that leads directly into a suction flask. McBryde, however, recommends the use of paraffin to seal the various joints which would include the joint between the filter surface and the vessel containing the liquid to be filtered.

The apparatus reported by McKinney[3] employs standard equipment used in connection with the Berkefeld type of filter with the addition of a "filter tube" to increase the efficiency of the filter. The recommended use of such joint-sealing materials as hot paraffin, however, as a means of adapting this filter tube to use with other than the Berkefeld type of filter, would prohibit the use of this apparatus for the work at hand. Furthermore, the Berkefeld alone is not reliable for the uniform production of sterile filtrates except under certain conditions. These filters having the same degree of fineness, vary widely in permeability, and for each filter it was found that there was a time limit for filtration beyond which sterile filtrates were not produced. It was decided, therefore, to devise a filtration apparatus for this work. The one here described proved to be completely satisfactory in the production of nearly a thousand sterile filtrates and has wide applicability.

THE APPARATUS AND ITS USE

The apparatus that was finally developed, after several attempts, and was tested by the production of nearly a thousand sterile filtrates, is illustrated in figure 1.

When properly set up the apparatus shown in figure 1 produces practically 100 per cent sterile filtrates from the same vacuum source and with no increase of time because the process of double filtration continues simultaneously. The first filtration is done by a Chamberland-Pasteur filter (A), of fineness "B," which connects to the T-shape side arm of the glass tube enclosing a Berkefeld filter (size 1 x 2 inches; fineness "w") through which a second filtration of the same juice is accomplished. The sterile filtrate thus continues through the system and is collected in the flask (D), which

[2] McBryde, C. N. Filtration experiments with *Bacillus cholerae suis*. U. S. Dept. Agr., Bur. Animal Indus. Bul. 113. 1909.

[3] McKinney, H. H. A method of increasing the efficiency of filter cylinders. Phytopath. 14: 585–586. 1924.

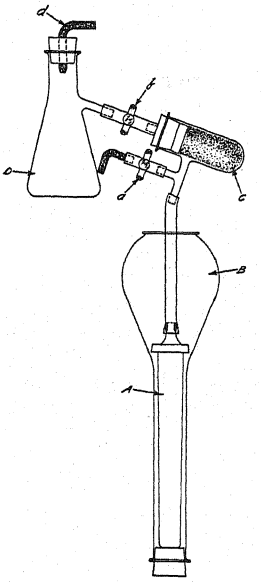


FIG. 1. Apparatus devised to obtain sterile filtrates of the juices of biological materials.

is of 250 cc. capacity but may be of any desired size. The flask (D) is an ordinary Pyrex Erlenmeyer flask into which a side arm has been sealed. The flask carries a bent-glass tube (d), plugged loosely with cotton to prevent possible contamination of the filtrates, and to this tube the source of vacuum is connected.

The assembly at (a) consists of a bent-glass tube filled with cotton and connects, through a short length of rubber tubing, to the T-shape side arm of (C). A screw clamp on the rubber tube is closed during operation. This assembly is used to obtain the filtrate contained inside the Chamberland-Pasteur filter (A) if needed. The filter is removed from the flask (B) and, while held in an inverted position, is shaken up and down rapidly to eject about 5 cc. of the contained liquid. This done, and with the filter maintained in an inverted position, the clamp at (a) is unscrewed to release the vacuum in the filter (A) very slowly. The sterile cotton in the bent-glass tube at (a) filters out contaminating organisms from the incoming air and the Berkefeld filter (C) holds the vacuum in the balance of the apparatus. After the vacuum in the filter (A) has been thus released the screw clamp at (a) is tightened immediately and the resulting vacuum causes the filtrate contained in the inverted filter (A) to run out and continue on through the apparatus to the flask (D). It may be necessary to repeat this procedure a second time.

The flask (B) is a Kjeldahl flask (500 cc.) with the neck stoppered and with a hole blown in the bulb end large enough to admit the filter (A). This flask is used to contain the juice to be filtered. It needs to be filled but once, the bulb acting as a reservoir. The neck of the flask fits closely around the filter (A) and maintains the filter at a high rate of efficiency due to the fact that only 25 to 30 cc. of juice in the neck of the flask will wet the entire surface of the filter. There are no joints that need to be sealed with agar or hot wax. All rubber tubing used in making connections is thick-walled ($\frac{1}{2}$ inch bore and $\frac{1}{8}$ inch wall) to withstand the external pressure during the process of filtration.

Before use, the assembled apparatus shown in figure 1, with the exception of the inverted Kjeldahl flask (B), is sterilized in an autoclave for 30 minutes at a pressure of 15 pounds. When setting up the apparatus but two operations are required, namely, to place the filter (A) into the inverted Kjeldahl flask (B) and to connect the apparatus to the source of vacuum through the bent-glass tube (d). During operation the clamp at (b) is open and the clamp at (a) is closed. Evacuation should be maintained at an equivalent pressure as near 30 inches of vacuum as possible.

When sufficient filtrate has collected in the flask (D) and it is desired to stop the process, (1) the clamp (b) is screwed down tightly, (2) the vacuum in flask (D) is very slowly released by breaking the rubber-tube connection on the bent-glass tube (d) after the source of vacuum has been shut off by a suitable clamp, and (3) the flask (D) is then disconnected from the remainder of the apparatus by removing the rubber tube from the metal-tube outlet of the Berkefeld filter (C).

The flask (D), which contains the sterile filtrate, after being thus disconnected, carries the bent-glass tube (d) filled with sterile cotton to prevent contamination of the filtrate. The side arm of this flask carries the short length of rubber tubing that is securely closed by the screw clamp (b). In this condition the flask (D) serves as a convenient and efficient means of transferring the sterile filtrate to such culture vessels or measuring devices as may be used. To accomplish this transfer, which preferably is done in a transfer room that has been steam-sterilized, (1) the rubber tubing on the side arm is removed, (2) the side arm is heated gently in a flame, and (3) the filtrate is poured out through the side arm into the culture vessels. This may be done either directly or into some suitable measuring device and from this into the culture vessels if it is necessary to measure the filtrate used.

This apparatus is adapted to being set up in series on the same vacuum line. A battery of apparatus so set up is illustrated in figure 2.

The efficiency of this apparatus through the use of the inverted Kjeldahl flask (Fig. 1, B) has been indicated. Its efficiency is increased by its

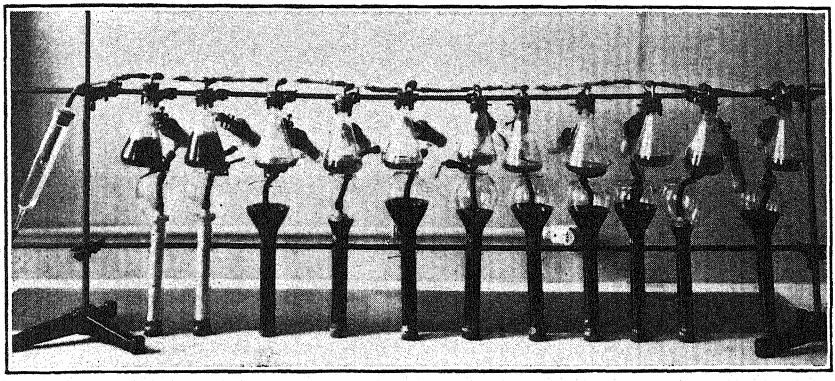


FIG. 2. Battery of filtration apparatus set up and in operation. The inverted Kjeldahl flasks (Fig. 1, B) are held in place by small copper wires twisted around the lower horizontal iron rod. Most of the weight of the apparatus is supported by the burette clamps around the necks of the flasks (Fig. 1, D). The vacuum line is extended by means of glass T-tubes connected together by thick-wall rubber tubing. All connections not subject to sterilization are shellacked.

general utility as a whole or in part. Any of the standard types of filters may be connected to the apparatus as the Chamberland-Pasteur filter (Fig. 1, A) is connected. Where only a single filtration is desired the filter may be connected directly to the flask (Fig. 1, D), thus taking advantage of the increased efficiency of filtration through the use of the inverted Kjeldahl flask, which, in its various sizes, is adapted for use with most filters.

The apparatus illustrated in figures 1 and 2 has been used to obtain more than a thousand sterile filtrates. The number of contaminated filtrates that developed has been negligible. At intervals it is necessary to replace the stoppers and rubber connections as repeated sterilization causes the rubber to lose its elasticity.

SUMMARY

An apparatus for the uniform production of sterile filtrates is described. It is of simple construction, of high efficiency as a medium of filtration, and of wide utility. The apparatus is readily adapted to the use of practically all types of filters without the use of such joint-sealing materials as agar or hot wax, which are so frequently recommended for such use.

A BRUSH TREATMENT OF MOLDY STAVES[1]

DOW V. BAXTER

The molding of staves has occasioned such severe losses throughout the South-Central and Southern States that mill owners have been forced to try various control treatments. Mill owners also have followed different prophylactic practices in the handling of the wood in the effort to lessen the possibilities of mold development. Some of these practices and the reasons for them are as follows: (a) By felling the timber in the winter when temperature conditions are not optimum for the rapid growth of mold, much molding of the staves is prevented. (b) Through immediate hauling of the logs or bolts to the mill, thus preventing the wood from being exposed to mold fungi in the woods. (c) By construction of dry foundations for bolts in the yard. (d) Through immediate sawing of all raw stock in the summer. Insect attack on timber felled in the summer causes serious losses in green wood. It is estimated that stave timber bolts and logs may be rendered worthless within three weeks after felling (from June to September) by insect attack. Felling timber in the winter avoids much of this loss. In the summer time bolts may become molded within a week. Rot, especially in gum, follows within a week of this blacking if optimum conditions for fungus growth are present. Most mill men cannot afford to have their yards filled with stored bolts during the spring and summer because of the rapid growth of mold during this season. (e) By the construction of suitable drying sheds which allow rapid seasoning of the staves. Since dry staves do not mold, these open sheds or buildings are ordinarily constructed so that they extend in a north and south direction. Usually the racks of staves are run east and west. This facilitates the drying out of the staves within a short time, after the wood has left the steam box.

Various chemical treatments have been tried with differing degrees of success in the effort to check molds and stains in wood.[2] Sodium fluoride, sodium bifluoride, sodium carbonate, and sodium bicarbonate have been used in treatments. Lime has been employed in the yards without any success. Mercuric chloride is probably one of the best antiseptics used to prevent mold in the steamed wood, but it cannot be applied to cooperage in which foodstuffs are shipped. Other chemicals are of doubtful value because they are ineffective or they may stain the wood or they may otherwise render it unfit for retention and shipment of foodstuffs.

[1] Contribution No. 14 from the School of Forestry and Conservation, University of Michigan.

[2] Howard, N. O. The control of sap-stain, mold, and incipient decay in green wood with special reference to vehicle stock. U. S. Dept. Agr. Bul. 1037. 1922.

Kiln-drying of stave wood has been found to be the most effective means of controlling mold, but the cost of installing a kiln is practically prohibitive even in the larger mills. The installation of a kiln in the smaller mills may represent an outlay of money greater than the cost of all the remaining equipment of the mill. There are additional objections to the drying of staves in kilns. Many mill operators object to the cost of fuel required to run a kiln. The wood which would be required to run the kiln is ordinarily sold and is depended upon as a source of revenue for the mill. Kiln-dried staves also are subject to warping. It is estimated that at least 10 per cent of the kiln-dried staves are so badly warped that they are rendered practically worthless. The remaining staves may be so badly warped that they are often degraded.

ECONOMIC IMPORTANCE OF MOLD IN STAVES

It has been estimated[3] that fungous stains in lumber cause an annual loss of over ten million dollars. Although mold has been considered of little importance in producing permanent discolorations in lumber, losses brought about by mold fungi in stave wood amount to thousands of dollars (Fig. 1, A and B). Little consideration has been given to moldiness in lumber because the molded surfaces can be so readily planed off in working up the wood. It can be seen readily that such small products as staves cannot be economically planed, even assuming that their dimensions would remain unaltered and that the staves themselves present a plane surface.

The losses caused by molds in staves vary with the kind of timber used and with the location of the mill. The observations recorded in this paper were made largely at the East Prairie Stave Company mill at East Prairie, Missouri. Red gum, the leading wood used in stave production, is most subject to molding in southeastern Missouri. Moldiness in elm, a species ranking next to gum in stave manufacture, places it second to red gum in susceptibility. Hackberry also may be placed in the same class with elm. It is, however, more subject to stain (blackening) than to mold. Sycamore is subject to a "rust," but this is not serious. Losses in maple staves are not appreciable, and ash very seldom molds. It is estimated that 60 per cent of the gum and hackberry staves and 10 per cent of the elm staves are damaged during the summer. When moisture and temperature conditions are most favorable for fungous growth (ordinarily between June and the last of September), mold may appear within three to five days after the staves are put out in the racks. These large losses are general in Arkansas, Mississippi, Tennessee, and Louisiana, as well as in southeastern Missouri.

In Mississippi and farther south the molding season is longer and greater losses are consequently suffered. It happens, however, that losses

[3] Hubert, E. E. The brown stains of lumber. The Timberman 27, No. 7. 1926.

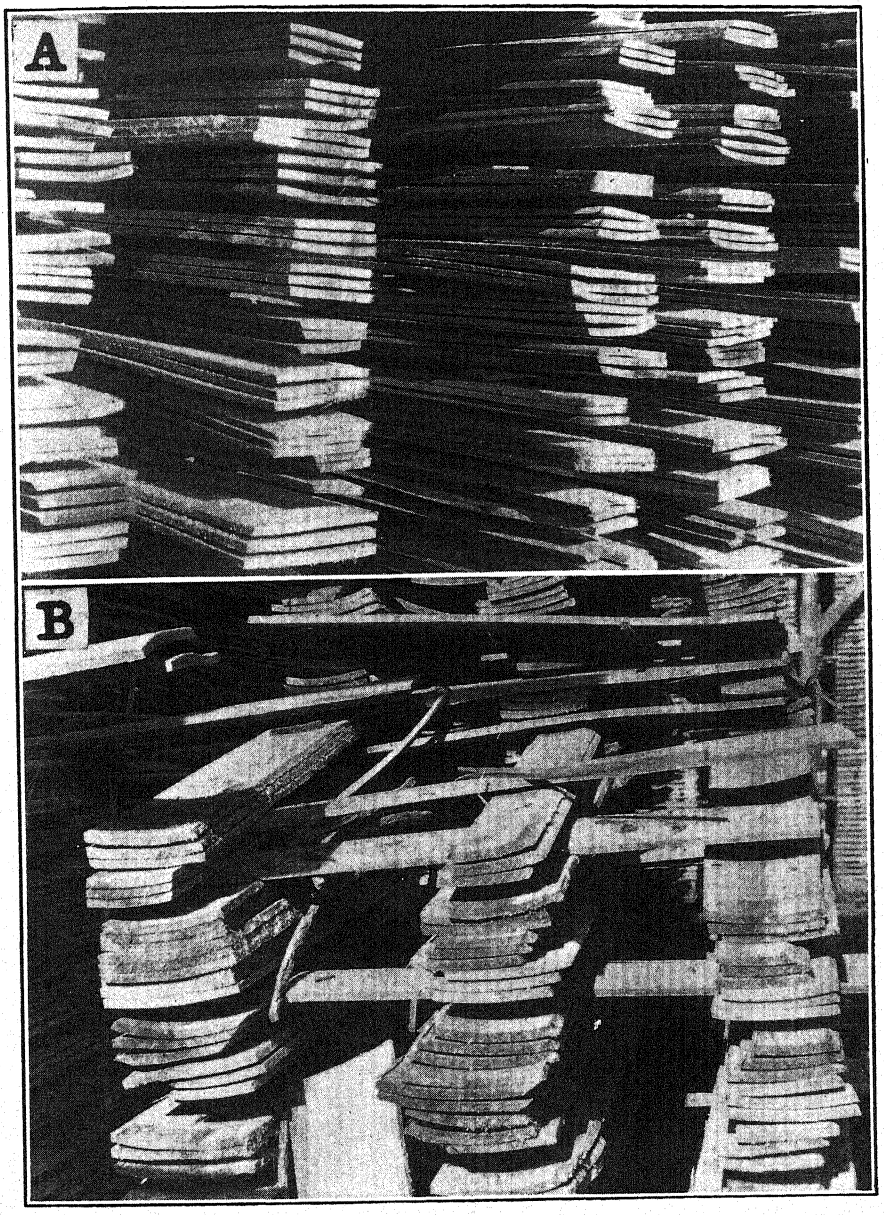


FIG. 1. A. Moldy staves, East Prairie, Missouri. (Roughened surface indicates moldy condition.) B. Moldy staves in stacks, East Prairie, Missouri. (East side.)

vary even within the same locality. At mills located only 30 miles apart, the loss caused by mold may be very severe in one mill yard and negligible in another. When losses do vary within a given region at different mills, differences in relative humidity are believed to be responsible. One mill yard, for example, at Water Valley, Mississippi, located in the river-bottom lands, where fog is frequent, is subject to mold; the other mill yard, at Batesville, is located at the edge of the river valley, back from the lower bottom lands, and is almost free from mold. The humidity of the atmosphere is undoubtedly lower in this latter locality.

Although molds, stains, and decay develop very rapidly in the bolts before they are converted into staves during the spring and summer months (Fig. 2), the rapid sawing of the bolts into stave wood ordinarily prevents

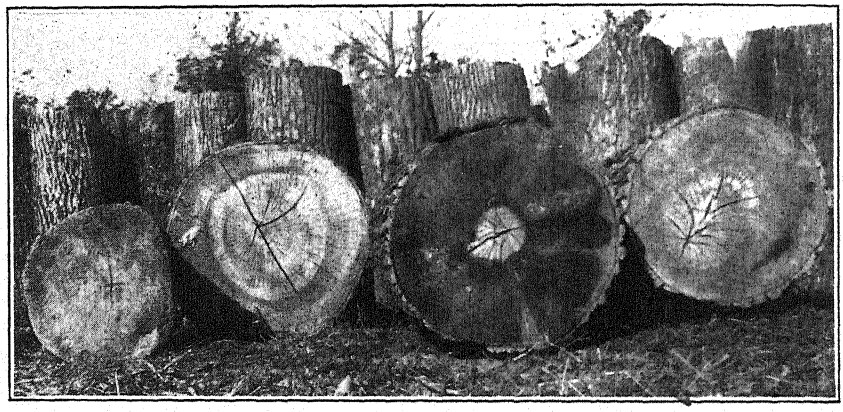


FIG. 2. Hackberry, elm, and two red-gum bolts in a yard at East Prairie, Missouri, for forty days. Bolts were bright when brought to the yard.

serious loss in this material. Unless the bolts are immediately converted into staves at this season, decay will follow the mold and the wood will be rendered worthless within a very short time.[4] However, as the mold itself develops on the ends of the bolts it produces little damage and causes little or no degrade in the product unless it penetrates deeply into the ends of the bolts. As the 32-inch bolts[5] are cut to 30 inches it is possible to saw off both ends. Therefore, bolts that have not been penetrated by mold to a depth greater than one inch on each end of the bolt are not damaged. Mold on the

[4] Boltwood decays sometimes very rapidly even during the winter and early spring. An exceptional example from Mississippi may be cited. Elm bolts cut in January and ricked in the open on high ground were moved to the mill during the last part of May. When the bolts were sawn into staves it was found that 100 per cent of them were worthless because of a "white rot." The trees were apparently sound at the time of felling.

[5] The bolts are procured in 32-inch lengths.

bolts and stave ends, however, offers sources of inoculum for the staves in the racks and may be the indirect cause for rapid mold development in the yards.

PRINCIPAL FUNGI CAUSING MOLDINESS IN STAVES

A number of fungi have been identified that cause decay in wood. Among these *Graphium* spp., *Aspergillus* spp., *Cladosporium* sp., and a number of species belonging to the genus Penicillium should be mentioned. In southeastern Missouri most of the damage is caused by species of the green mold, Penicillium. The so-called red mold causes very little damage.

THE BRUSHER

When stock cannot be treated chemically, either because of the partial or complete ineffectiveness of the treatment or because the product is to be used for shipping of foodstuffs, the operator suffers tremendous loss. When

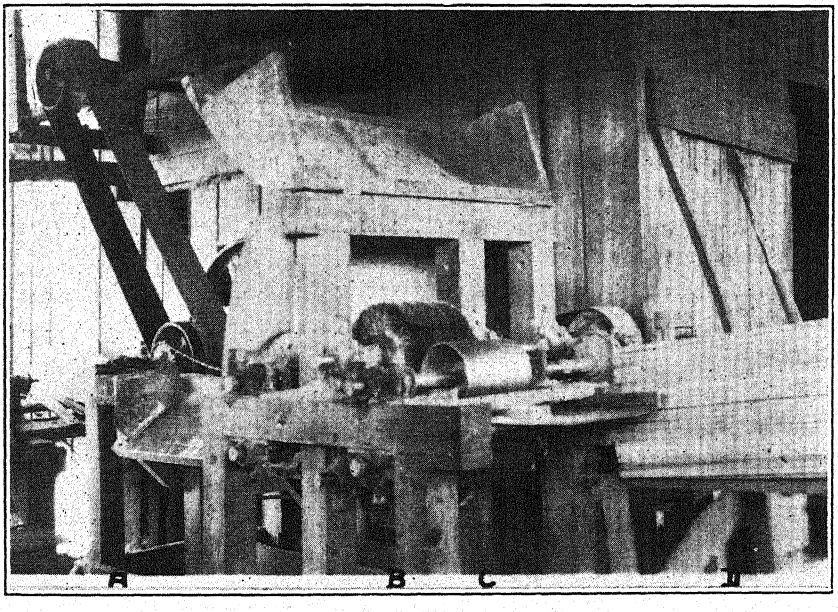


FIG. 3. Brushing machine, East Prairie, Missouri. A, drive shaft; B, steel brush; C, steel rollers; D, stave rack.

operating conditions prohibit the installation of a kiln or when the product warps badly because of such rapid seasoning brought about by kiln drying, use of the kiln in immediate practice does not seem to offer results of the most practical value.

It is believed that a brushing machine, designed by Mr. D. D. Baker,[6] of the East Prairie Stave Company, offers a method for salvaging much of the damaged stock in the mill yards. After the moldy staves have passed through the jointer they are moved to the brushing machine for treatment. Following is a description of this machine.

The brushing machine consists (Fig. 3) of two 10-inch, stiff steel-wire brushes, figure 3, B, so set that the brush tips barely touch. Two sets of steel rollers, C, are placed in front and also behind the two brushes. The speed of the machine is so set that the brushes have a peripheral speed with a ratio of 15 to 1 to the roller. This speed insures a sufficient brushing for the staves as they pass between the steel brushes. The brushes will take the staves at this speed as fast as one can feed them into the machine. The power for the brusher is furnished by the power plant used for generating the steam for the boxes in the mill.

SALVAGED STAVES

Brushed staves are placed in a grade known as "brushed number 1" (Fig. 4, A and B). This grade commands the same market price as bright number 1 staves. At present prices, moldy staves are worth $10.00 a thousand, while brushed red-gum staves are worth $13.00 to $13.50 a thousand. This saving is an important item to the mill operator when it is realized that, counting the time of the man needed to untie the bundles and feed the machine, the cost of brushing approximates only 40 cents a thousand. Table 1 indicates the amount of salvaged staves realized from this additional operation in the mill. The estimates given in this table are for the salvaging of staves by brushing during the molding season when conditions for mold growth are optimum.

TABLE 1.—*The salvage of moldy staves by the brush treatment*

Species (arranged in order of their degree of susceptibility to mold)	Percentage of moldy staves that can be salvaged by the brush treatment
Red gum	Approximately 40 per cent can be salvaged during the spring and summer. Sapwood gum begins to decay within 30 days
White elm	Practically 100 per cent will brush. This figure is particularly gratifying when one considers that elm is second only to gum in importance in the stave industry
Sycamore	Practically 100 per cent will brush

[6] The writer wishes to express his indebtedness to Mr. D. D. Baker for his cooperation and help during his visits at this mill.

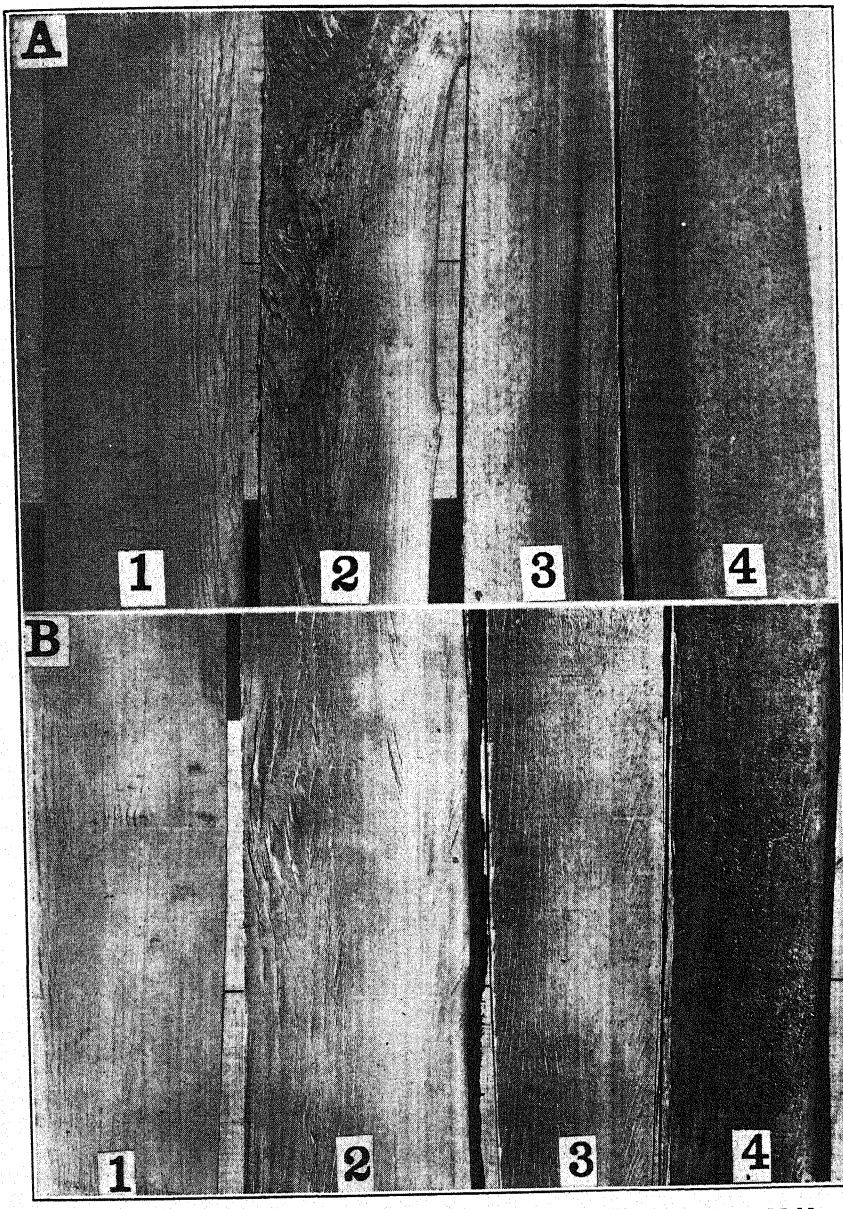


FIG. 4. A. White-elm staves, East Prairie, Missouri. 1, Bright No. 1; 2, Moldy elm (rust); 3, Brushed No. 1; 4, Moldy elm. B. Red-gum staves, East Prairie, Missouri. 1, Bright No. 1; 2, Blue-stained gum, stain in wood before made into staves; 3, Brushed No. 1; 4, Moldy gum.

As the staves are dry after they leave the brushing machine, no loss from molding occurs after the staves have been bundled. Blue stains, which usually appear in the woods before the bolts reach the yards, "fleck" stains, caused by Penicillium, itself, and "rust" are not removed by the brusher. The fleck stains left after brushing, however, do not prevent the staves from being placed in the brush number 1 grade.

CONCLUSIONS AND SUMMARY

1. Although stains, and particularly blue stains, in lumber have attracted much attention and have been the subject of numerous and long-time investigations in both Europe and the United States, very little information on the molding of wood is at hand. This is in part due to the fact that the molds generally have been regarded as superficial growths, readily removable by planing. The damage caused to staves by molding, however, amounts to an annual loss of thousands of dollars and a waste of valuable timber, as molds on staves cannot be removed by planing. It is, furthermore, difficult and often impossible to give them effective chemical treatment. Although staves are dried frequently in the kiln, there are several objections also to this treatment for stave timber.

2. A mechanical method is described by which staves can be brushed between steel brushes. This method insures a salvage of practically 100 per cent of the molded white-elm and sycamore staves. The number of red-gum staves that may be salvaged depends somewhat upon the season. Approximately 40 per cent of the red-gum staves, however, may be salvaged by brushing. These figures are believed to be particularly gratifying, as red gum is the leading wood used in stave production and white elm ranks second only to gum. The stave brusher here described is a very simple machine and can be installed without much added expense to the operator. The operating expense of the stave brusher approximates 40 cents a thousand staves.

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MODIFIED PROCEDURE WITH THE KEITT SINGLE-SPORE METHOD[1]

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While using the single-spore method described by Keitt[2] the writer has tested various modifications and eventually developed a procedure that has proved exceptionally useful in isolating single-spore strains of many fungi, including species of Sclerotinia, Fusarium, Alternaria, Helminthosporium, Phoma, Chaetomella, Rhizopus, and Rosellinia. The details of the procedure are not new; but the combination not only retains the advantages of the original Keitt method in that it is simple, requires little special equipment, and is applicable to many kinds of spores, but also has the further advantages of greater rapidity and ease of operation.

CHANGES FROM THE ORIGINAL METHOD

The procedure outlined by Keitt is as follows: A dilution series is poured in petri dishes, in one of which a suitably located spore is selected under the microscope, usually after germination has rendered the sporeling easily visible. A special needle with a hollow, cylindric end (Fig. 1, *b*) is then used to cut out a disk of agar which includes the spore. This disk is transferred by a second special needle, with a flattened and twisted tip, to another petri dish for further growth.

The important change has been the substitution of a streak culture, in a petri dish of set agar, for the dilution series previously used. In plates of dilution series, spores were distributed at various depths vertically and in unknown positions horizontally throughout the agar. Microscopic search for suitably isolated spores was, therefore, exceedingly tedious. With the streak isolation, on the other hand, spores are distributed only at a definite depth in the agar and in streaks of definite width and need be sought only along these streaks. All the inoculum is to be found in one plane of observation with the 16 mm. objective. This has greatly reduced the time and energy necessary to find spores far enough apart in the agar to allow removal of individual spores in agar blocks.

A straight chromel[3] needle with a broad, rounded tip (Fig. 1, *a*) has been substituted for the needle with the twisted tip formerly used for the

[1] Published with the approval of the Director as Technical Contribution No. 88 of the Texas Agricultural Experiment Station.

[2] Keitt, G. W. Simple technique for isolating single-spore strains of certain types of fungi. Phytopath. 5: 266–269. 1915.

[3] Chromel (or nichrome) wire of B. & S. gauge No. 24 has been found excellent for the needles mentioned.

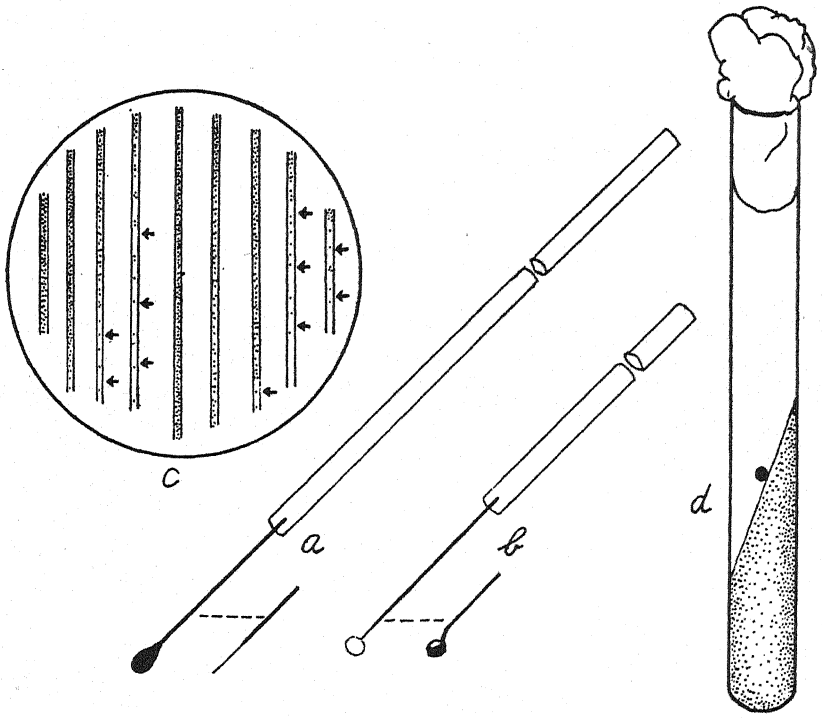


FIG. 1. Single-spore procedure. *a*, Top and side views of needle with spatulate tip; *b*, top and oblique views of needle with cylindric tip; *c*, diagram of distribution of spores in a streak culture, the arrows indicating regions in which spores might be selected; *d*, position of agar disk, containing the selected sporeling, after transfer to agar slant.

transfer of the agar disk. The straight needle is preferable when the disk is to be inverted as described below and also is useful in inoculating the streak plate. To prepare the two needles, the tips of two wires are first hammered flat. One tip is twisted to form the cylinder shown in figure 1, *b*, and the wire is mounted in a short handle 8 to 10 cm. long. The edges of the flattened tip of the other wire are smoothed, if necessary, with a file, and this wire is then mounted in a longer handle.

A further change is the use of agar slants rather than petri-dish cultures for the sporelings selected. By inverting the agar disk as it is transferred and placing the disk at the edge of the slant, it is possible to obtain almost as clear vision of the selected spore and of growth from it as if the transfer were to a petri dish. Advantages of use of the tube are the greater freedom from accidental contamination, especially when isolating slow-growing organisms, and the greater convenience of storage of tube cultures.

THE REVISED PROCEDURE

Preparation of the streak plate. The agar should be suitable for growth of the organism at hand but need not be extraordinarily clear. Potato-dextrose agar and oatmeal agar, filtered through absorbent cotton, have, after final sterilization, rather abundant, fine, granular precipitates, which have not hindered frequent use of these media. The granules aid in focusing on the surface of the agar, without obscuring vision of the spores scattered along the streaks. The consistency of the agar is of importance. It should be firm enough to permit the flat tip of the inoculating needle to make a definite scar in the preparation of the streaks but not be so dense as to make difficult the cutting out of the disks. The quantity of agar to be used per plate varies with the thickness of the glass bottom of the petri dish, since microscopic observation is through this to the opposite surface of the agar. Microscopic observation becomes more difficult as the depth of the agar increases, while manipulation of the agar blocks becomes easier. With the standard (95 mm. diameter) petri dish, 6 to 8 cc. of standard 1 per cent or 1.5 per cent agar per dish has usually been satisfactory.

The petri dish is flamed and the agar poured while both are hot, using the customary aseptic precautions. After the agar has cooled, a liberal amount of inoculum is taken up on the spatulate-tip needle, which is then drawn across the agar in a series of four or five parallel streaks. Two series of streaks are made in each dish when sufficient inoculum is available. Pycnidia, perithecia, or apothecia may conveniently be crushed in the droplets of water which condense on the cover of the dish and the resulting suspension then used in preparing the streaks. The broad tip of the needle is held flat against the surface of the agar and pressed into it hard enough to leave a mark visible macroscopically. This is done to aid in locating the streaks later, and such markings in the agar do not interfere with microscopic examination, except perhaps when working with unusually minute spores. The needle is rotated in the fingers very slightly at the beginning of each stroke across the plate, so that a portion of fresh surface touches the agar. As a result, each streak begins with a more or less crowded mass of spores but thins down to spores separated by increasingly greater intervals (Fig. 1, *c*). It is along the latter part of the streaks that isolated spores are found.

Selection of single spores. Except with large, dark spores it is best to defer selection until 16 to 24 hours after preparation of the plates to allow enough growth to facilitate observation as well as to prevent selection of nonviable spores.

The petri dish is examined through the bottom without opening it. First the 16 mm. objective is used to run along the streaks until an isolated spore-

ling is found. This is examined further with the 8 or 9 mm. objective to insure that only a single spore is at the center of the observed growth. If the sporeling appears satisfactory, a dot of ink is placed (with a fountain pen) directly over the sporeling. The petri dish can now, without danger of losing the place, be moved around to ascertain whether the space around the sporeling is actually free of other growth. After this, an ink ring is drawn around the original ink dot to indicate the clear working space around the spore. The plate is then turned with the top up and the agar disk cut around the spore.

Cutting the agar disk. The cylindric-tip needle is flamed, cooled, held with its center exactly above the ink dot, plunged down rapidly and immediately withdrawn, thus making a circular incision around the spore. The ink dot may now be removed from the bottom of the petri dish; and the cylindric agar disk now marked off may be examined microscopically, through the bottom of the dish, to insure that the sporeling selected is actually included in the disk and that no other growth is included in it.

Transferring the agar disk. The flat-tip needle is slid under the disk, which is then raised and transferred to an agar slant. To facilitate observation, the disk is placed with its upper surface against the glass at the edge of the slant but part way down the slant to insure sufficient moisture for growth. Since the spore was placed originally on the surface of the agar, it will now be quite close to the glass of the tube and may be examined usually not only with the 16 mm. objective but also with the 8 mm. objective. The rest of the disk should also be examined, as additional spores that were not observed in the previous view from below may possibly be found now in this surface view.

Further check on the monosporous nature of the culture is obtained by rapid microscopic examination of the agar disk during further incubation. All growth should be from the single selected sporeling.

SUMMARY

The following procedure has been found useful for single-spore isolation:

(1) A streak culture is prepared in a petri dish of set agar.

(2) The dish is inverted and a suitably isolated sporeling is selected under the microscope and its position indicated with a dot of ink.

(3) The dish is turned up again and a disk of agar including the sporeling is cut out.

(4) The agar disk is transferred to the edge of an agar slant.

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A RHIZOCTONIA DISEASE OF SWEET ALYSSUM[1]

PAUL E. TILFORD

In July, 1927, the writer's attention was directed to a disease of sweet alyssum, *Alyssum odoratum*, occurring in a garden in Wooster, Ohio. Sweet alyssum is commonly used as a border around beds of annual flowers. The plants are set close together, thus forming a dense, low, hedge-like border.

SYMPTOMS AND ETIOLOGY

The disease started in the lower portion of the plants, where the leaves and stems lay on the ground. These diseased parts first appeared water-soaked and looked as if they had been scalded. The disease progressed rapidly, rotting the leaves and causing them to shrivel up (Fig. 1). Defi-



FIG. 1. Sweet alyssum plant affected with Rhizoctonia, on left; healthy plant, on right.

nite lesions appeared on the stems and usually the portion of the plant above the lesion was killed. Leaves on the upper part of the plant appeared normal unless a large lesion occurred lower down on the stem. In no case were lesions noted on the main stem below the level of the soil.

[1] Published with the permission of the Director of the Ohio Agricultural Experiment Station.

Early in the morning or during rainy periods an abundance of a cob-web-like mycelium was visible on the diseased plants. Microscopic examination of this fungus showed it to have the morphological characteristics of Rhizoctonia. A Rhizoctonia was isolated from the diseased stems and leaves and grown in culture. Healthy plantings of sweet alyssum were inoculated with the organism and after 18 days (during this period there was considerable rain) the plants were badly diseased. The organism was reisolated.

Peltier[2] has reported a Rhizoctonia on sweet alyssum growing in pots in the greenhouse. He states that small lesions were formed on the stems near the ground. Teng[3] has recently described a Rhizoctonia disease of Lobelia which in many respects is similar to the disease of sweet alyssum reported here. Also, the brown-patch disease of grass, originally described by Piper and Coe,[4] shows similar characteristics.

The sweet-alyssum Rhizoctonia was grown on oatmeal and potato-dextrose agar at different temperatures and compared with other Rhizoctonias isolated from potato, aster, and chrysanthemum. Agar was poured in petri dishes to a depth of about 5 mm. Small bits of inoculum were transferred from 3-day-old tube cultures to the center of the agar plates. The plates were then placed inside paper bags and put in constant-temperature incubators ranging from 5° C. to 30° C. at 5-degree intervals. The plates were examined at 12-hour intervals and measurements made of the radial growth. Two plates were used with each organism at each temperature, and the two measurements were averaged.

The organisms isolated from aster and chrysanthemum grew at about the same rate. The sweet alyssum organism grew a little faster at 30° and at 15° than the other two. All three grew faster than the potato organism. This was especially true at 30°. All four organisms grew faster on oatmeal agar at 10° and 15° than on potato-dextrose agar at these temperatures. No growth occurred in any case at 5° C. The experiment was discontinued after 168 hours. The rate of growth of the four organisms on potato-dextrose agar is shown in figure 2.

The color of the mycelium of all four organisms ranged from white at the lower temperatures to dark brown at the higher temperatures. Zonation occurred only in the case of the organisms isolated from aster, chrysanthemum, and potato. The sweet-aylssum organism formed abundant brown to black sclerotia which seemed to pile up on each other, thus forming large irregular sclerotia.

[2] Peltier, G. L. Parasitic Rhizoctonias in America. Ill. Agr. Exp. Sta. Bul. 189. 1916.

[3] Teng, S. C. Rhizoctonosis of Lobelia. Phytopath. 19: 585–588. 1929.

[4] Piper, C. V., and H. S. Coe. Rhizoctonia in lawns and pastures. Phytopath. 9: 89–92. 1919.

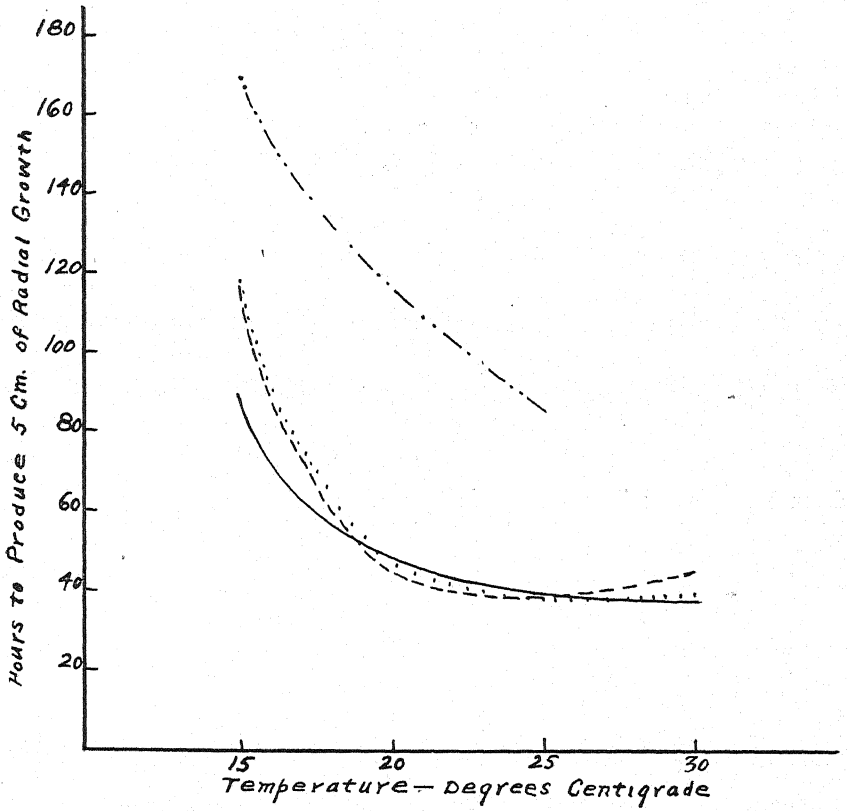


FIG. 2. Effect of temperature on rate of growth of different strains of *Rhizoctonia solani* on potato-dextrose agar

- ·· ___ ·· Isolated from potato.
- _____ " " sweet alyssum.
- ..... " " chrysanthemum.
- – – – – " " aster.

While the sweet alyssum Rhizoctonia varies considerably from the potato Rhizoctonia, yet there are no morphological or physiological characteristics to suggest that it should not be classed as *Rhizoctonia solani* Kühn. Both Monteith and Dahl[5] and Peltier[6] have reported great variations in the growth characters of *Rhizoctonia solani* when isolated from different hosts and grown in culture. Peltier reports similar variations when different strains, isolated from the same host, are compared.

[5] Monteith, J., Jr., and A. S. Dahl. A comparison of some strains of *Rhizoctonia solani* in culture. Jour. Agr. Res. 36: 897–903. 1928.

[6] *Loc. cit.*

CONTROL

Plantings of sweet alyssum in which the disease was very serious were sprayed with 4–6–50 Bordeaux mixture on July 25, 1927. A second treatment of Bordeaux was made on August 1. Parts of the planting were left nonsprayed to serve as checks. After 18 days the disease was completely checked on the sprayed plants. They were sending out new shoots and growing well. The disease had progressed in the nonsprayed plants and at this time they were practically dead. Sweet alyssum can be thoroughly sprayed without drenching the soil and no ill effects should follow from an accumulation of copper in the soil, as is the case when Bordeaux is used to control brown patch.

SUMMARY

1. A Rhizoctonia disease of sweet alyssum is described.

2. The organism is compared with *Rhizoctonia solani* Kühn isolated from potato and with Rhizoctonia isolated from aster and chrysanthemum. The sweet alyssum organism is a strain of *Rhizoctonia solani* Kühn.

3. The disease can be controlled by spraying with Bordeaux mixture.

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NOTES ON *PHYLLOSTICTA RABIEI* ON CHICK-PEA

RODERICK SPRAGUE

INTRODUCTION

Following the same general lines as those pursued in the recent work on diseases of legumes caused by Ascochyta,[1] the writer was able through materials and cultures sent by Dr. D. Atanasoff from Bulgaria to determine that the common disease of chick-pea, *Cicer arietinum* L., caused by *Phyllosticta rabiei* (Pass.) Trotter, is distinct from any Ascochyta on legumes. This has been clearly shown, in part at least, by Trotter,[2] but his work does not seem to be widely accepted. In the light of recent studies by the writer and others it is thought advisable to publish this note.

Although Trotter found no trace of septate spores in the herbaria he examined, this was not true of the writer's study. Without reviewing the literature on the subject it may be mentioned that many texts list the chick-pea as a host for *Ascochyta pisi* Lib. Trotter examined all of the historic collections and concluded he was dealing with a Phyllosticta. An examination of several French and Italian collections and a study of the fungus on inoculated plants convince the writer that faint septations, up to about 5 per cent of the total, are commonly, though not always, present. These septations usually are visible only with the aid of an oil-immersion lens. Many spores show a constriction at their equator. In spores from inoculated plants a gradual formation of the oil-like contents into two separate masses is noted with the occasional constriction, sometimes resulting in a cross wall.

COMPARISON OF SYMPTOMS

A comparison of mature aerial lesions produced by *P. rabiei* with those produced by *Mycosphaerella pinodes* and *A. pisi,* respectively, is given in table 1.

COMPARISON OF CULTURAL CHARACTERS

Tissue transfers on potato-dextrose agar (1 per cent dextrose), in test tubes under ice-box conditions, show a grey, felty growth with a small amount of a cream color, fluffy mycelium. Pycnidia develop in profusion at the top of the slant or along the sides next to the glass and exude pale pink spore masses. At first the medium turns opalescent and later becomes nearly black. These cultural characteristics contrast rather strikingly with

[1] Sprague, R. Host range and life history studies of some leguminous Ascochytae. Phytopath. 19: 917–932. 1929.

[2] Trotter, A. Riv. Patol. Veg., n. s. 9: 105–114. 1918.

TABLE 1.—*Comparison of mature aerial lesions produced on legumes by Phyllosticta rabiei, Mycosphaerella pinodes, and Ascochyta pisi, respectively*

Name of fungus	Characteristics of lesions			
	Color	Zonation	Border	Pycnidia
P. rabiei	Mottled brown to deep tan	2–3 (rarely more) wide irregular bands	Vague to prominent (red)	Very obscure
M. pinodes	Dark brown	Numerous	Vague	Obscure
A. pisi	Light tan	None	Prominent (red brown)	Prominent

the pink, fluffy mycelia and carrot-red pycnidial spore masses produced under similar conditions by *A. pisi.*

Cultures of *P. rabiei* made by spreading spore suspensions in water on the surface of pea-soup agar show many acervuli and pycnidia exuding small flesh-color spore masses that run together and often nearly cover a plate. Scarcely any fluffy mycelium is developed. Often interesting tentacle-like patterns of pycnidia radiate from several central areas on a plate.

In similarly produced cultures, *A. pisi* forms brighter, pink to carrot-red spore masses. Often these are fewer and larger and accompanied by a certain amount of evanescent, fluffy mycelium.

Compared with *M. pinodes* under similar conditions both of tissue transfers and of plate cultures from spore suspensions, *P. rabiei* shows a far greater tendency to fruit and has a lighter mycelium with little, if any, of the dark brown color of *M. pinodes.*

RESULTS OF ARTIFICIAL INOCULATIONS

The method of inoculation was the same as that used in former work, namely, young plants were sprayed with spore suspensions in water. Incubation was in a spray chamber and further incubation in a moist location. Reisolations were made where lesions were obtained.

The chick-pea organism showed negative results on several varieties of the garden pea, *Pisum sativum,* on *Vicia villosa, Lathyrus odoratus, Dolichos lablab, Lens ervum,* and two varieties of *Phaseolus vulgaris.* On the chick-pea water-soaked lesions appeared after five days. A few days later all but the most woody portions of the aerial parts of the host were dead and brown. Pycnidia developed in great profusion. This virulent blighting is the same type as seems to be common in Europe where

its scourge-like nature has attracted scientists since the days of Theophrastus and Pliny.

Inoculations on chick-pea with *A. pisi,* isolated from the garden pea, showed a doubtful tip injury to the chick-pea. In these inoculations strains of *A. pisi* both from the United States and Bulgaria were used.

In recent work by the writer[3] it was shown also that *M. pinodes* could cause severe foot-rot injury to the chick-pea, as could *A. pinodella* L. K. Jones. In the present study one inoculation on the aerial parts of chick-peas gave doubtful infection. The amount of work done was insufficient to determine definitely if *M. pinodes* would severely injure the aerial parts of the chick-pea.

DISCUSSION AND CONCLUSIONS

Everything considered, including morphology, it is clear that *P. rabiei* is distinct from *A. pisi.* Its relation to *M. pinodes* is closer. In shape of pycnospores, characteristics of pycnidia, and in pathogenicity, the two have some similarities. However, the nearly total lack of septation in pycnospores, differences in incubation period, difference in virulence on aerial parts, differences in host symptoms, the absence of a perfect stage in *P. rabiei,* and rather striking differences in cultural characters on agar make the two distinct. That *M. pinodes* may be found on the chick-pea in nature is highly probable, especially as a parasite producing foot rot.

The position of the chick-pea organism in the genus Phyllosticta is not so definite as Trotter assumed it to be. He and the writer agree, however, that there may have been some past genetic connection with Ascochyta. That the disease of chick-pea caused by *P. rabiei* must be dealt with as a problem distinct from that of those diseases caused by *A. pisi* and *M. pinodes,* respectively, is definitely shown.

This study was made at the University of Cincinnati subsequent to work for the doctorate under the advisory direction of Dr. O. T. Wilson. The writer is also indebted to Mr. Harry Muegel for translation of the extensive Italian literature.

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[3] *Loc. cit.*



BOOK REVIEW

Seymour, Arthur Bliss. Host index of the fungi of North America. 732 pp. Harvard University Press, Cambridge, Mass. 1929.

This book constitutes another outstanding contribution of Harvard University to those phases of botany especially pertinent to plant pathology. Its value is derived not only from the fact that a large part of a lifetime of labor has been devoted to its preparation, but from the skill and thoroughness with which a vast literature has been handled. It is the seemingly impossible dream of many a youthful mycologist brought to realization. Its dedication to the memory of William Gilson Farlow is at once a worthy tribute and a reminder of earlier associations. It recalls the fact that the Farlow herbarium and library must always continue of inestimable value to American mycology. It will also remind some of us that modern plant pathology in America began with the return of Farlow from de Bary's laboratory in the mid-seventies to inaugurate his epochal studies on black knot and other American plant diseases at the Bussey Institute. The titles mycologist and plant pathologist were synonyms in the early days of our profession. This usage is met today in British literature. Perhaps this may permit the reviewer, as one who has favored the modern trend toward the physiological approach in phytopathological researches, to stress the attendant danger that our younger professional recruits neglect their mycology.

The present work should therefore be a frequent reliance to the present-day plant pathologists, as was the first edition, the Farlow-Seymour Provisional Host Index of over 40 years ago, to those then exploring our fields for parasitic fungi. Pertinent statements from the first and this second index merit quotation. From the first edition: "Believing that an approximately complete list of our parasitic species and their hosts would aid materially in the advance toward a more accurate study of our mycological flora and would lessen the amount of indiscriminate species making which has already become a serious evil, the present index, the result of work extending over several years, has been prepared for publication." From the second edition: "The primary object of this work is to indicate, so far as recorded in the literature, for each host plant, all the fungi known to grow upon it and for each fungus all the hosts upon which it grows." In addition to their main purposes as indicated above, these scrupulously exact lists will, perhaps for years to come, serve as the most universally reliable American guide in mycological nomenclature. Their convenience will be felt in three respects—inclusive synonymy, correct spelling, and usage in

species capitalization. The author explains that he has decapitalized species names "whenever possible." The conservative principle of using capitals for those derived from personal and generic names is, however, followed. The reviewer believes that American botanists should follow the practice of the zoölogists in decapitalization of all species names. But for those who do not, this list will serve as a reliable basis of good usage. That it is practically impossible, however, for anyone to follow the rules of partial capitalization consistently is shown by the occasional deviations—*e.g.*, p. 368, "*Labrella Pomi*," "*Cylindrosporium pomi*."

The inclusiveness of this index and the labor involved in its preparation are obvious when one notes that its 717 pages list some 80,000 names of hosts and fungi. The growth of a generation in American mycology is further indicated by the fact that the earlier edition of 213 pages contained only 23,000 or somewhat over one-fourth as many names. The usefulness as well as magnitude of the undertaking is again brought home if one examines the length of the list of fungi which may be recorded as occurring on almost any widely distributed cultivated plant. For example, those on the common apple (*Pyrus malus*) occupy nearly 5 pages and include some 340 named organisms. For each of some 75 of these, 2 to 8 names are listed as synonyms. As to the vexed questions of further unrecorded synonymy the author wisely indicates that "it is not the function of this work to solve botanical problems," although it must "present problems to be solved by others." "Each fungus name stands on its own merits, and the compiler has not changed it by substituting or adding a name more correct or better understood." The author regretfully explains that this list does not follow the plan of the earlier work in arranging all synonyms in chronological sequence, thus giving the history of the name.

The reviewer heartily endorses the statement that "no pains have been spared to make this index as complete and accurate as possible." This book should, therefore, be on the reference desk of every mycological and phytopathological library and laboratory. The author and his institution, Harvard University, deserve our congratulations and thanks on the completion and publication of this monumental undertaking. May we hope that it will sometime be followed by the complementary publication of the completed Bibliography of North American Fungi of which the first volume under Farlow's leadership and the patronage of the Carnegie Institution raised such great anticipations.—L. R. JONES, University of Wisconsin, Madison, Wis.

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THE EFFECT OF AIR TEMPERATURE ON THE PATHOGENICITY OF RHIZOCTONIA SOLANI PARASITIZING GRASSES ON PUTTING-GREEN TURF[1]

LAWRENCE S. DICKINSON

INTRODUCTION

The observing green keeper recognizes a correlation between weather conditions and the appearance of the disease known as large brown patch. He frequently speaks of "brown patch weather." Little or no practical use, however, is made of these observations. The active cause (*Rhizoctonia solani* Kühn) of the disease is known, but little consideration has been given to air temperature as a possible factor in its control.

Rhizoctonia solani is the fungus which causes the disease commonly called large brown patch. It attacks most grasses and is particularly injurious to putting-green turf. It develops a fine white cobweb-like mass of mycelium on or within the turf. The hyphae of this mycelium enter the grass leaf (usually through the stomata) and break down the cells, causing the leaf to shrivel and become brown. Only in the very severest attacks do the hyphae enter the grass stems or injure the grass roots. The fungus forms sclerotia, or resting bodies, that are able to withstand unfavorable conditions. These sclerotia are found in most cultivated soils as dark brown, membranous, persistent bodies of corky texture and visible to the naked eye. They are about 2 mm. in diameter or larger. During weather unfavorable to the development of the fungus, sclerotia may fall upon the ground or remain on the grass leaves and stems until the return of conditions favorable to their growth. They are hardy to the usual extremes of temperature and, during the winter, are preserved in the soil or upon the dormant grass leaves and stems. The mechanical destruction of the sclerotia is impracticable, and their corky texture makes them resistant to fungicides.

Scientists (2, 4, 5, 7, 11) have shown that the ability to induce certain parasitic fungous diseases is conditioned on a specific range of soil temperature, together with other factors, and that there are maximum, optimum,

[1] Massachusetts Agricultural Experiment Station Contribution No. 105, 1930.

and minimum temperatures for the development of a given fungus. Jones (3) and Richards (9) found that Rhizoctonia is most virulent to potatoes at soil temperature of 15°–21° C. "with a distinct and rapid lessening of the diseased condition at higher temperatures" up to 24° C., above which development was only of minor significance. They also found that the sclerotia at a soil temperature of 15°–18° C. produced the greatest number of hyphae. On the other hand, Rolfs (10) and Peltier (8) found that high temperature (30° C.), together with too much or too little moisture, determined to a large degree the virulence of various strains of Rhizoctonia. Monteith (6) demonstrated that Rhizoctonia develops best at 83° F. when cultured in petri dishes stored in constant-temperature chambers. However, almost no growth showed at 50° F. or 61° F. and only a third as much at 94° F.

A review of the literature previously cited showed that nearly all scientific studies on Rhizoctonia have been made with potatoes as a host and that the temperature studies have been made with the fungus growing on potatoes in soils kept at various constant temperatures or by culturing the fungus on a nutrient medium in petri dishes stored at various constant temperatures. It is to be noted that none of the investigators has reported on the appearance of large brown patch or the development of the mycelia of Rhizoctonia in their relation to changing air temperatures.

As the sclerotia and mycelia of Rhizoctonia generally are prevalent in most soils and large brown patch appears only occasionally, it would seem that the disease is not so much dependent upon the presence of sclerotia or mycelium as it is on favorable environmental conditions.

In turf the sclerotia are subjected more to air than to soil temperatures, since most of them are on the surface of the soil or on parts of the grass. This surface soil of short-grass putting-greens is more quickly affected by a change in atmospheric temperature than surface soil under taller grasses of lawn turfs. Furthermore, it has been observed by agrostologists, pathologists, and green keepers that putting-greens are more susceptible to brown patch than lawns or fairways. On putting-greens the mycelium frequently appears at the greens edge and not in the adjacent taller grass of the same species. Thus, it would seem, the sclerotia develop mycelia more favorably where the atmospheric temperatures exert their greatest influence.

OBSERVATIONS LEADING TO LABORATORY STUDIES

During the past five years the writer has obtained many data concerning these environmental conditions which favor the appearance and development of the large-brown-patch disease. The sources of these data were personal observations, contact with green keepers, and professional journals.

SUMMARY OF DATA FROM GREEN KEEPERS AND PROFESSIONAL JOURNALS

Because the reported observations have been made in the morning after development of large brown patch had started, investigators and green keepers have been led to believe that the disease is nocturnal (6, 1). On the other hand, in the test plots at the Massachusetts Agricultural College, it has appeared in both mid-afternoon and late afternoon, following certain environmental conditions. Also, it is quite probable that this disease, appearing on many greens in the late afternoon, was not observed until the following morning.

The large brown patch may appear on any putting-green comprising grasses that are susceptible to the disease, but it appears definitely and more often on putting-greens where the soil is low and poor and the air drainage is defective. The disease also appears following afternoon watering on hot days and subsequent to a rapid drop in air temperature from 80°–95° F. to 60°–70° F. Putting-greens that have been rapidly cooled by a chilling wind, rain, or the settling of cool air over low areas are very frequently attacked by the disease.

The disease appears as a mild attack or fails to appear during cool periods, continued hot periods, following evening watering or dewless nights, or on putting-greens that receive little or no artificial watering.

Humidity appears to influence the appearance of the disease only as it influences the air and surface soil temperature. Under certain temperature conditions the disease will appear on upland and exposed putting-greens, as contrasted to the lowland and enclosed greens.

FIELD OBSERVATIONS

Three experimental putting-greens at the Massachusetts Agricultural College are being employed in this investigation. The greens are located side by side, and each one has a uniform soil of medium heavy loam. The greens are exposed to direct sunlight until late afternoon when the western halves are in shadow. Air circulation is unrestricted except for a distant wind-break on the south and east sides. Each green is seeded or planted in ten-foot squares with varieties and strains of putting-green grasses. (Metropolitan, Washington, Flossmore, Virginia, Columbia, and Rockford strains of creeping bent; Kernwood, Newport, and Fall River strains of velvet bent; Rhode Island, Colonial, South German, Cocoos, and Fiorin bent; Red and Chewings fescue.) The mean elevation above sea-level is 242 feet. The areas are Green I—1,000 square feet, Green II—4,200 square feet, Green III—3,000 square feet.

Three years ago it was noted that the appearance of large brown patch regularly followed a rapid fall of air temperature, provided the cooling

was immediately followed by a rise in air temperature. It also was noted that the fungus appeared from three to three and a half hours after the rise in air temperature began, and, if its growth were destroyed at once by attrition, such as poling, by vigorously sweeping a bamboo pole back and forth on the surface of the green, or by fungicides, there was no injury to the turf. It was observed also that the growth of the mycelium was more rapid when the cooling was accompanied by rain. The practical significance of such conditions was realized and led to further studies during the past two years. The environmental conditions have been so accordant that each appearance of the disease and its degree of severity could be forecast.

Definite Data of Air Temperature as Correlated with Appearance of Large Brown Patch. To obtain temperature data, a Weather-Bureau instrument shelter was placed in the center of the putting-green area. In the shelter were installed a standard thermograph and corrected maximum and minimum thermometers. The thermograph is checked twice daily against the thermometers and is one foot above the turf. The thermometers are one foot higher. In presenting these data it is realized that the air temperature outside the shelter has a wider range than on the inside.

The humidity records were obtained during 1928 by means of a sling psychrometer rotated breast-high above the grass. Because the humidity records were inconsistent at the time of the appearance of the disease and because of the greater degree of humidity in the dew-laden grass little value could be given to the humidity readings. They were not recorded in 1929. Further meteorological data, such as sunshine, cloudiness, and rainfall, are obtained from the college station, located five hundred yards to the west of the experiment greens.

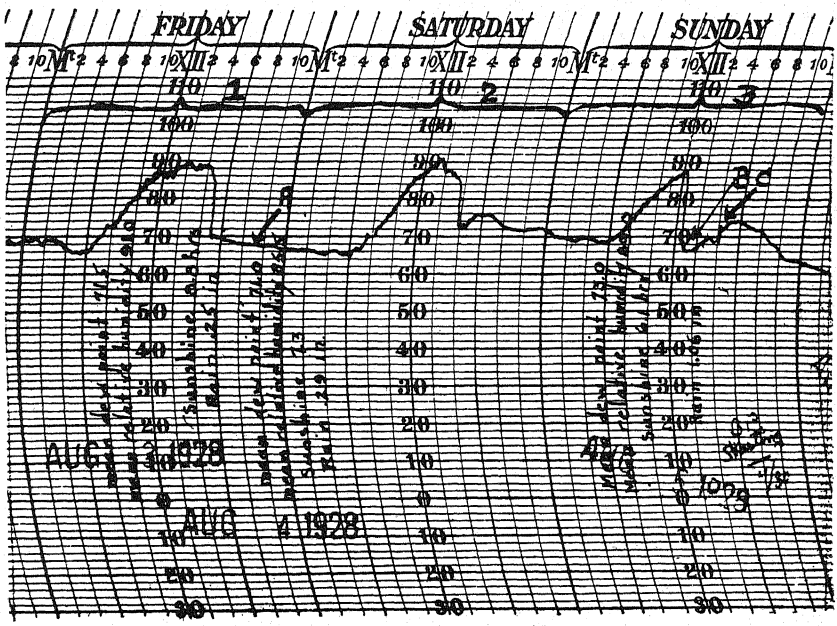
The records also showed that if the optimum temperature range (64°–68° F.) is reached from a lower temperature, the air must remain from eight to ten hours at a nearly constant temperature within the optimum range to secure growth of sclerotia.

Summary of Field Observations. A summary of the evidence secured from field observations to date clearly indicates a close correlation between air temperature and the appearance and development of *Rhizoctonia solani.* Two phenomena must be given consideration, the growth of short mycelia from the sclerotia and the development of these mycelia from inactive organisms dependent on the sclerotia for food to independent parasitic organisms. (Field and laboratory observations repeatedly showed that the sclerotia grew short mycelia (10–15 mm.) soon after they had been chilled. These short mycelia remained inactive until environmental conditions favored their further development or they were destroyed.)

Development of Mycelia from Dormant Sclerotia

Representative Thermograph Readings Selected from Three Seasons' Records

Record A



Under field conditions, if the optimum temperature was reached from a higher temperature, growth did not occur unless the change was very rapid.

1. No growth of sclerotia was expected, since abrupt drop in temperature failed to reach the optimum range (64°–68° F.) until four hours after the fall, indicated as A.
2. No growth of sclerotia was expected, since the optimum range was not reached.
3. Very severe attack was expected and did occur, since the sudden drop in air temperature was to the optimum range, indicated at B. Spread of mycelium began at 4:30 p. m. (73° F.), indicated at C. Immediate poling completely checked the mycelium already developed. On a nonpoled plot the spread of mycelium was noted until the temperature fell to 70° F.

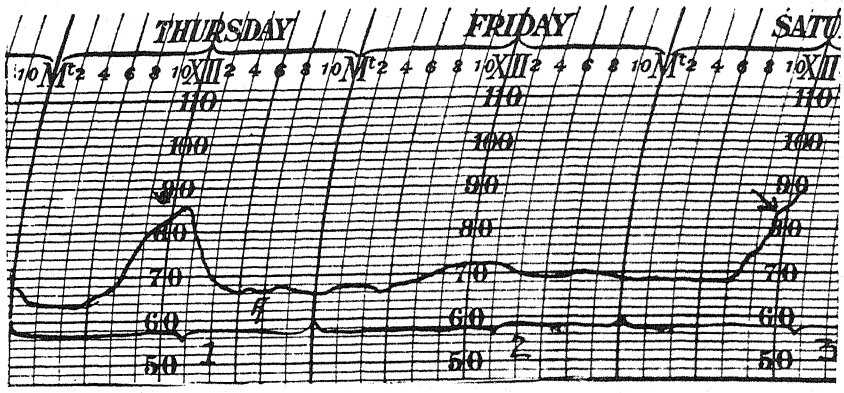
The optimum temperature range for the growth of the short mycelia from the sclerotia appears to be between 64° and 68° F., and the required duration of temperature within such range is variable.

The mycelia will appear in from 30 minutes to one hour after the chilling to the optimum stage.

The development of the fungus to the point of becoming actively parasitic is concurrent with a rise in air temperature.

Virulence appears to require a rather rapid rise in air temperature from 64°–68° F. to between 80°–85° F. Parasitism commences at about 73° F. and ceases at 90° F. If the air temperature falls below 62° F. the mycelia on the sclerotia are destroyed.

Record B



After short growth from the sclerotia the mycelia will remain inactive until environmental conditions favor their development, or they may be destroyed by attrition or excessive heat or cold.

1. It is to be noted in the instance illustrated that on Thursday growth of the sclerotia should be expected at between 7 and 8 p. m. (A). The temperature was too low for rapid mycelial development but, realizing that there were many sclerotia covered with very short mycelia, the green was not disturbed.
2. On Friday no attack was noticeable to the ordinary observer because the mycelia had not developed sufficiently to be visible as cobweb-like masses.
3. Saturday observations at 7, 8, 9, 10 and 11 a. m. found no apparent attack of the fungus. Observation at 11:45 a. m. found that the mycelia had developed so rapidly that the usual circular diseased patches were very noticeable.

Following the occurrence of short mycelial growth from the sclerotia further development of the organism probably is dependent on high humidity as well as air temperature.

No reference has been made to other recorded meteorological data, such as rainfall and sunshine, because they failed to show vital significance to the mycelial development except as they affected the air temperature. The mycelium develops from the short nonparasitic stage more rapidly on moist grass than on dry, and it makes little difference whether the moisture on the grass is obtained as dew, rainfall, or artificial watering. Yet, several attacks have been observed on dry grass.

The observations recorded are practical evidence of the soundness of the opinion that a rapid chilling of the air temperature is a very important factor in the appearance of *Rhizoctonia solani* on grasses. In the appearance of the disease, moisture seems to be of more consequence as a modifier of the air temperature than as a direct factor in the growth of the sclerotia.

It is to be noted these records showed that during the "brown patch season" there were only 5 per cent. of the days in which environmental conditions favored growth of the sclerotia and subsequent mycelial development. By being able to forecast the appearance of the disease and confine

TABLE 1.—*Records of field observation after sufficient data had been collected to indicate favorable and unfavorable conditions*

	1928	1929
Date of first record	July 1	May 27
Date of last record	Oct. 31	Oct. 1
Number of days observed	123	123
Number of days favorable to virulent attack	7	5
Number of attacks occurring on days unfavorable to virulent attacks	0	0
Number of days disease observed	7	5
First attack	July 9	June 25
Last attack	Aug. 17	Aug. 22

the use of fungicides to such times, a green keeper can effect a considerable saving in the cost of golf-course maintenance.

Practical Adaptation of Observations. To test the practical adaptation of the observations previously reported, two methods of control have been used: (1) Fungicides as preventives and controls and (2) attrition by severe poling. Nontreated and nonpoled plots were used as checks. The results when summarized show:

1. A fungicide, if applied only when environmental conditions indicate its need, is inexpensive, a complete protection from attack, and seldom injurious to the turf.

2. A fungicide as a control, if applied very soon after the sclerotia produce the short mycelia, prevents further development of the mycelia, is inexpensive, and is seldom injurious to the turf.

3. Attrition by poling very early each morning (a few hours after sunrise) destroys the activity of mycelium that may have developed during the preceding 24 hours. Control by daily poling has been 60 per cent effective on the experimental plots. Complete control by this method is not possible because favorable environmental conditions for the growth of the sclerotia occasionally occur in late morning or during the afternoon, a fact overlooked by the majority of green keepers.

4. Attrition by thorough poling soon after the sclerotia germinate affords approximately 100 per cent control, is inexpensive, and causes only a roughing of the turf.

PETRI-DISH EXPERIMENTS[2]

Preliminary Laboratory Experiments. The object of the laboratory experiments was to check the field results by placing the sclerotia under more or less controlled conditions.

[2] These experiments were performed at the Boyce Thompson Institute for Plant Research, Inc., at Yonkers, New York. To Dr. William Crocker, Director of the In-

A medium was prepared by placing a small amount of corn meal in each of six Erlenmeyer flasks and covering it with distilled water. The flasks were then plugged with cotton and sterilized in an autoclave.

Rhizoctonia solani sclerotia and mycelia were transferred to the Erlenmeyer flasks, the transfer being made in a sterilized transfer case. The sclerotia developed mycelia which, in turn, formed sufficient sclerotia to be used as supply colonies for future experiments.

In each series of experiments an equal amount of potato-dextrose agar was placed in each petri dish as a medium. All transfers of Rhizoctonia sclerotia were made with a sterilized needle and in each colony there was placed approximately the same number of sclerotia.

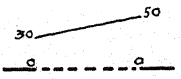
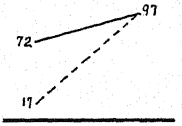
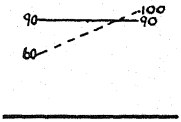
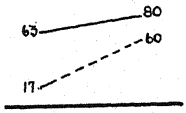
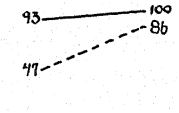
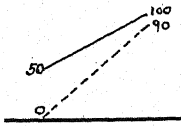
Measurements of mycelial growth were obtained by holding the petri dishes to a strong light and carefully tracing the outline of the mycelial area on cross-section paper divided 10 x 10 to the half inch. Thus, each unit of measurement represents 1/400 of a square inch.

TABLE 2.—*Table showing average mycelial growth in units from one colony of sclerotia in petri-dish cultures*

No. of exp.	Conditions to which sclerotia were subjected	Sclerotia chilled to optimum temperature range		Sclerotia not chilled to optimum temperature range (Checks)	
		Hours after chilling period		Hours after chilling period	
		3½	17½	3½	17½
I.	Average mycelial growth of all sclerotia used in the experiment	24.3	438	6.3	225.8
II.	Maximum temperature 30° C. Minimum temperature 25° C. (Except chilling period)	25.8	449.8	10.3	347.3
III.	Minimum temperature above 30° C. (Except chilling period)	22.8	364.5	11.2	319.8
IV.	Maximum temperature below 25° C. (Except chilling period)	0	40.5	0	35
V.	Maximum temperature 35° C. Minimum temperature 25° C. (Except chilling period)	21.1	480.2	10.2	411.3
VI.	Minimum temperature above 35° C. (Except chilling period)	13.75	43.75	0	16

stitute, and his associates, the writer expresses sincere appreciation for their helpful advice and criticisms; also, for the use of the equipment at the Institute. Acknowledgment is also made for the valuable assistance rendered by Dr. William H. Davis of the Department of Botany at the Massachusetts Agricultural College.

Summary graph of petri-dish experiments with Rhizoctonia solani

No. of exp.	Conditions to which sclerotia were subjected	Percentage of sclerotia[3] showing mycelial growth	
		3½ hrs. after chilling	17½ hrs. after chilling
I.	Sclerotia chilled to optimum temperature range and warmed above prechilling temperature		
II.	Sclerotia chilled to optimum temperature range and warmed to prechilling temperature		
III.	Sclerotia chilled to optimum temperature range and warmed to a lower temperature than that before chilling		
IV.	Sclerotia chilled to optimum range but warmed to a maximum temperature above 30° C. either before or after chilling		
V.	Sclerotia chilled to optimum temperature range but warmed to a maximum temperature of 30° C. either before or after chilling		
VI.	Sclerotia chilled to optimum temperature range but warmed to a maximum temperature of 25° C. either before or after chilling		

[3] ———— Sclerotia chilled to optimum range (17°–20° C.)
– – – – – – Sclerotia not chilled to optimum range (checks).

The examination of the dishes was made at room temperature and the notes taken as rapidly as was consistent with accuracy. The air-temperature changes were obtained by placing the dishes in standard temperature chambers regulated to the desired temperatures. (19°, 20°, 25°, 30°, 35°, 30° C.)

At intervals all dishes were placed in the 30° C. chamber to provide a most favorable growing condition.

Table 2 indicates the following: (1) Twenty degrees C. appears to be too cool for vigorous mycelial development. (2) Mycelial development is retarded by a rise in air temperature to 35° C. or above. Total mycelial growth from sclerotia at start of experiment, 1,229 units. Total growth after having been warmed to 35° and 39° C., 966 units, showing a decrease of 29 per cent. (3) Chilling appears to be very essential for the growth of sclerotia if the air temperature is warmer than 35° C. (4) Mycelial growth in all cases was very weak at the time of the first observation and could be destroyed very easily by fungicide or attrition.

POT EXPERIMENTS

Growth of Sclerotia on Earth and Grass. The object of this experiment was to obtain data as to the growth of the sclerotia of *Rhizoctonia solani* when placed in field conditions but subjected to temperature control.

Cultures of Metropolitan creeping bent, in two-inch flower pots, were used for the experiments on turf, and loam in two-inch pots was used for the experiments on earth. Small flower pots were necessary because the temperature chambers were small.

In each series of experiments approximately equal numbers of sclerotia were transferred from a culture in an Erlenmeyer flask to the grass or soil. The transfers were made with a sterilized needle and the sclerotia placed on the surface of the loam and on the grass about one-half inch above the earth. Pots were marked: 1, *grass damp;* 2, *grass dry;* 3, *earth damp;* 4, *earth dry.* Four pots, one of each number, formed a series, and all temperature changes were made with series and not with individual pots.

In each experiment all pots were kept three hours in an equalizing or starting temperature, just as in the case of petri-dish experiments. Those scheduled for chilling were taken directly from the first chamber to the cool chamber (18°—19° C.) and kept there from 30 to 45 minutes and then placed in warmer chambers as scheduled and held there from 14 to 15 hours.

Observations were made at each changing of chambers and at the end of the 14-hour period. Measurement of growth was made on a comparative basis. The pot showing the greatest development in each experiment was credited with 20 points, credit to the other pots being in proportion to their relative development. Growth of the sclerotia was recorded by actual count of colonies showing any mycelial growth. At the close of each experiment complete control of the disease was effected by the mechanical disturbance of the mycelia.

Summary of the Pot Experiments. 1. The growth of the sclerotia was hastened and increased if the sclerotia were cooled to the optimum range (64°—68° F.) (17.5°—20° C.) for 45 minutes.

2. Sixty per cent of all sclerotia that were chilled showed short mycelial growth at the end of the chilling period.

3. No sclerotia kept at a constant temperature showed mycelial growth at a time corresponding to the end of the chilling period.

4. Mycelial development was greatly increased in those instances where the sclerotia had been chilled.

5. Chilling to the optimum temperature range appears necessary to the growth of the sclerotia under practical conditions, because normal air temperatures do not remain constant at 25° C. or 30° C. (77° or 86° F.) long enough to promote the growth of the sclerotia.

TABLE 3.—*Record showing growth of sclerotia and development of mycelia under damp and dry conditions*

Pots	Percentage of sclerotia showing mycelial growth	Comparative mycelial development
Grass damp	68	27
Grass dry	53	18
Earth damp	83	45
Earth dry	71	41
All grass	60	22
All earth	77	42
All damp	76	35
All dry	63	30

The results shown in the above table are similar to those noted in field observations, namely:

1. Moisture is favorable to the growth of the sclerotia and development of the mycelia but is not of vital significance.

2. Sclerotia grow better and the mycelial development is greater when the sclerotia are on the earth than when they are on the grass.

The laboratory results, though not entirely consistent, indicate that there is a scientific value in the field observations that a rapid chilling of the air temperature is a very important factor in the appearance of *Rhizoctonia solani* on grasses and that further scientific investigation is warranted.

PRACTICAL SIGNIFICANCE

The practical siginficance of this investigation is that it indicates:

1. Favorable air temperature is vital to the growth of the sclerotia of *Rhizoctonia solani.*

2. There are two phenomena to be considered: The growth of short mycelia from the sclerotia and the development of the mycelia after such growth takes place.

3. That it is possible to forecast accurately the appearance of the fungus, and therefore limit control measures to such times, thus effecting a great saving in cost.

4. It is practicable to check effectively the development of the mycelia by mechanical attrition, and, if attrition is applied soon after growth of the sclerotia takes place, control is as complete as when fungicides are used and with less injury to the turf

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AN ABERRANT PHYSIOLOGIC FORM OF PUCCINIA TRITICINA ERIKS.[1]

C. O. JOHNSTON[2]

INTRODUCTION

The recent demonstration by Newton and Johnson[3] of the occurrence of mutation in *Puccinia graminis* Pers. is of great interest and importance to the cereal-rust investigator. It gives material weight to an important theory on the origin of physiologic forms, although the mutant types observed by them did not differ from the parental types in their infective capabilities. The mere establishment of the fact of mutation under controlled conditions is of great importance even though the mutants apparently were not new physiologic forms. It seems reasonable to suppose that if the rust fungus can mutate for such a heritable character as color, it also can mutate for the equally heritable character of physiologic specialization. It seems, therefore, that in mutation we may find a possible origin of physiologic forms, as well as in the phenomenon of heterothallism, demonstrated by the classic experiments of Craigie.[4,5,6]

The mutations observed by Newton and Johnson[7] occurred in *Puccinia graminis tritici* and involved the characters of color, viability of urediniospores, and spore dimensions. Similar mutations have not been described in the other cereal rusts. Since there is, however, a strikingly close analogy between these fungi in morphological and physiological characters, we may expect to encounter them in the course of future experiments. In the writer's experiments with the leaf rust of wheat, *Puccinia triticina,* mutation has not been observed to occur under controlled conditions. An aberrant form has been studied, however, which may have arisen through mutation. It was particularly interesting in that it differed from other known forms, principally in length of incubation period and in spore color.

[1] Contribution No. 266 from the Department of Botany and Plant Pathology, Kansas State Agricultural College, Manhattan, Kansas, in cooperation with the Office of Cereal Crops and Diseases, U. S. Dept. of Agriculture.

[2] Associate Pathologist, Office of Cereal Crops and Diseases, U. S. Dept. of Agriculture.

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[7] *Loc. cit.*

COMPARATIVE GREENHOUSE STUDIES

Greenhouse studies have been made at Manhattan, Kansas, for four years on physiologic specialization in *Puccinia triticina*. More than 200 collections of leaf rust have been cultured during the progress of these studies. Many physiologic forms have been found that differed in their ability to infect certain varieties of wheat, but all save one were almost identical in color, length of incubation, and certain morphologic characters.

In June, 1927, a collection of leaf rust was received from Mr. P. B. Dunkle, superintendent of Texas Substation No. 6, at Denton, Texas, which has proved to be markedly different from other known forms of *Puccinia triticina* in several characteristics. The rust was collected by Mr. Dunkle because it occurred on Texas No. 3015–63, a pure-line selection of Mediterranean wheat known to be highly resistant to leaf rust. The writer had visited the Denton station on May 10 and at that time the upper leaves of Texas No. 3015–63 were practically rust-free, although they exhibited marked flecking. Nearly two weeks later, on May 23, Mr. Dunkle noted the presence of numerous small, light-color uredinia on that variety and sent specimens to Manhattan for examination.

The rust, upon receipt at Manhattan, was given the culture number 199 and transferred to seedlings of Kanred, C. I. 5146, then being used as a susceptible variety upon which collections of leaf rust were cultured and increased in quantity. One week later there were no signs of infection having taken place, but the inoculated seedlings were not discarded. At the end of ten days a few flecks had appeared, but no uredinia had developed. This was an interesting situation in view of the fact that a number of other collections being cultured at the same time were showing flecks at the end of 4 to 6 days and developing uredinia in 8 to 10 days. In fact, most cultures of leaf rust of wheat then under investigation were fully developed at the end of 12 days. In the case of culture No. 199, however, no uredinia appeared until the 15th day after inoculation, and full development was not reached until much later.

Since several other cultures of leaf rust were being grown simultaneously in the greenhouse with culture No. 199, it was deemed advisable to repeat the studies under very carefully controlled conditions. The original observations were made on a culture grown in a cloth cage. These cages were made of a good grade of muslin stretched over a frame one foot square and a foot tall, the top being covered by a pane of glass. Such cages have been used in greenhouse rust studies at Manhattan and have proved to be very effective in preventing mixtures of cultures and forms.

When culture No. 199 was transferred for further study, however, the transfers were made from 6 uredinia taken at random from the original

culture to wheat seedlings grown under aseptic conditions under lantern globes, the tops of which were sealed with a thin layer of cotton between squares of cheese cloth. The spores from each uredinium were transferred to seedlings in a separate pot so that 6 single-pustule cultures were thus established. Bulk transfers also were made at the same time to seedlings kept in the muslin cages. The variety Harvest Queen rather than Kanred was used as the host in these studies because it is susceptible to more physiologic forms of leaf rust than Kanred. For comparison, transfers of physiologic form 9 were made at the same time and in identically the same way as those of culture No. 199. All cultures were watched carefully during the incubation period and the dates for flecking and eruption of the uredinia, as well as other characters, were noted. The results of this experiment are given in table 1. It is clearly shown that not only is the

TABLE 1.—*Comparison of the incubation and developmental periods and size and color of uredinia of culture No. 199 and physiologic form 9 of Puccinia triticina on Harvest Queen wheat in the greenhouse. Inoculated April 15, 1928*

Culture or form No.	Bulk or single pustule	Number of days from inoculation to			Size of uredinia	Color of uredinia
		Flecking	Sporulation	Full development		
199	bulk	10	14	23	small	light orange
199–1	s. p.	11	14	23	"	" "
199–2	s. p.	10	13	22	"	" "
199–3	s. p.	10	15	23	"	" "
199–4	s. p.	10	15	23	"	" "
199–5	s. p.	10	15	23	"	" "
199–6	s. p.	10	14	23	"	" "
p.f. 9	bulk	5	8	13	large	orange red
9–1	s. p.	5	8	13	"	" "
9–2	s. p.	5	8	13	"	" "
9–3	s. p.	5	8	13	"	" "
9–4	s. p.	5	8	13	"	" "
9–5	s. p.	4	7	13	"	" "
9–6	s. p.	5	8	13	"	" "

incubation period longer for culture No. 199 than for physiologic form 9, but also that the time required for the culture to reach its full development is longer. Furthermore, it became evident that the spores of culture No. 199 were much lighter in color than those of physiologic form 9 and the uredinia were smaller in size. The latter point might suggest to some investigators that these peculiarities were due to the resistance of the host

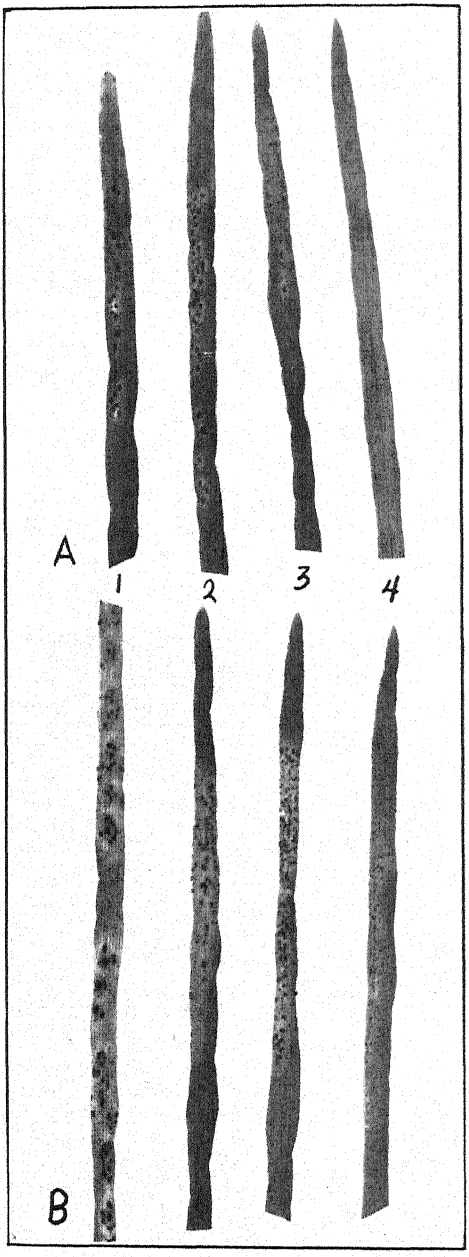


FIG. 1. Leaves of Harvest Queen wheat collected March 28, 1929, inoculated with (A) culture 199, and (B) physiologic form 9 of *Puccinia triticina*. 1. Inoculated March 2, 1929. 2. Inoculated March 5, 1929. 3. Inoculated March 14, 1929. 4. Inoculated March 20, 1929.

to that culture of leaf rust. It is true that in one form of resistance there is a reduction in the size of the uredinia. Small uredinia due to this type of resistance, however, are invariably accompanied by necrosis around the uredinia, and there is very little extension of the incubation period. Small or even minute uredinia on highly resistant varieties develop nearly as quickly as large normal uredinia on susceptible varieties. In the case of culture No. 199, however, there was no necrosis of the tissues surrounding the uredinia and the length of the incubation and developmental stages was strikingly longer than for physiologic form 9.

The difference in the length of incubation period is clearly illustrated in figure 1, showing eight leaves of Harvest Queen wheat, the lower four inoculated with physiologic form 9, and the upper four with culture 199. Each vertical pair of leaves was inoculated on the same date progressively later from left to right. Pair No. 1 was inoculated on March 2, No. 2 on March 5, No. 3 on March 14, and No. 4 on March 20, 1929. All leaves were collected on March 28. In Pair No. 4 eight days after inoculation the leaf inoculated with physiologic form 9 showed uredinia just breaking through the epidermis, while no uredinia had developed on the leaf inoculated with culture 199. Pair No. 3 showed physiologic form 9 well developed at the end of 14 days, while culture 199 was just rupturing the epidermis, only a few of the uredinia being completely uncovered.

Studies on the comparative length of the incubation period of culture 199 and physiologic form 9 were continued in the greenhouse during the fall and winter of 1928–1929. Nine different transfers of both cultures

TABLE 2.—*Comparison of the incubation periods of physiologic form 9 of Puccinia triticina and culture No. 199 on Harvest Queen wheat in the greenhouse, 1928–1929*

Date of Inoculation	Physiologic form 9		Culture 199	
	Number of days from inoculation to			
	Flecking	Sporulation	Flecking	Sporulation
Oct. 23	5	8	13	19
Nov. 12	6	8	11	14
Feb. 5	6	8	10	14
Mar. 2	4	7	8	12
Mar. 5	5	8	9	13
Mar. 9	4	7	9	12
Mar. 20	4	7	6	12
Mar. 26	4	7	5	12
Mar. 29	4	8	7	14
Average	4.6	7.5	8.6	13.5

were made to the variety Harvest Queen at different times during the season and the dates on which flecking and sporulation was first observed were recorded. These data are summarized in table 2. It is very evident that the average interval from inoculation to the beginning of sporulation was nearly twice as long for culture 199 as for physiologic form 9.

A set of the varieties having differential reaction to leaf rust was inoculated with culture 199 on April 20, 1929. These are the varieties used in the identification of physiologic forms and their reaction could be expected to shed some light on the question of whether the long incubation period of culture 199 was due to resistance of the host variety. A similar set was simultaneously inoculated with physiologic form 9. Notes were secured on the length of the incubation and developmental periods in each case (Table 3). It will be noted that the incubation period of form 9 was only

TABLE 3.—*Comparison of the incubation and developmental periods of two cultures of P. triticina on varieties of wheat having differential reaction in the greenhouse. Inoculated April 20, 1929*

Variety	C. I. No.	Culture or form No.	Number of days from inoculation to			Size of uredinia	Color of uredinia
			Flecking	Sporulation	Full development		
Malakof	4898	199	8	12	22	small	light orange
		p.f. 9	5	7	12	large	orange red
Mediterranean	3332	199	8	13	23	small	light orange
		p.f. 9	5	8	13	minute	orange red
Democrat	3384	199	8	13	23	small	light orange
		p.f. 9	5	8	13	minute	orange red
Hussar	4843	199	9	13	24	small	light orange
		p.f. 9	5	7	12	"	orange red
Webster	3780	199	8	12	22	small	light orange
		p.f. 9	5	7	11	large	orange red
Norka	4377	199	8	12	22	small	light orange
		p.f. 9	5	8	11	large	orange red
	3756	199	9	13	23	small	light orange
		p.f. 9	5	8	13	"	orange red
	3778	199	9	13	23	"	light orange
		p.f. 9	5	8	13	"	orange red
	3779	199	8	13	23	"	light orange
		p.f. 9	5	7	11	large	orange red
	3747	199	8	12	22	small	light orange
		p.f. 9	5	7	12	large	orange red
Kanred	5146	199	8	13	23	small	light orange
		p.f. 9	5	7	12	large	orange red

slightly longer on the four resistant varieties, Mediterranean, C. I. 3332, Democrat, C. I. 3384, C. I. 3756, and C. I. 3778, than on the other selections. The incubation period and time required to reach full development on all varieties were much longer for culture 199 than for physiologic form 9.

TABLE 4.—*Comparison of the length of incubation periods of several cultures of Puccinia triticina in the greenhouse, Manhattan, Kansas, 1929*

Variety	C. I. No.	Culture No. 369–6		Culture No. 308		Culture No. 367		Culture No. 199	
		Number of days from inoculation to							
		Flecking	Sporulation	Flecking	Sporulation	Flecking	Sporulation	Flecking	Sporulation
Malakof	4898	5	8	5	8	5	...	9	14
Mediterranean	3332	5	9	5	9	5	8	8	16
Democrat	3384	5	9	5	9	5	8	8	16
Hussar	4843	5	8	5	9	5	9	9	16
Webster	3780	5	8	5	...	5	...	9	16
Norka	4377	5	8	5	8	5	...	9	16
	3756	5	8	5	...	5	9	9	14
	3778	5	8	5	8	5	8	8	16
	3779	5	8	5	9	5	8	8	14
	3747	5	9	5	...	5	...	9	16
Kanred	5146	5	8	5	8	5	8	8	14
Average		5	8.2	5	8.5	5	8.2	8.5	15.2

It is true that some of the varieties showed some necrosis and other signs of resistance to culture 199, but that did not seem materially to affect the length of the incubation period. This experiment was also repeated in the greenhouse in 1929 (Table 4). Here again it will be noted that the average interval from inoculation to sporulation for culture 199 was nearly twice that of the three other cultures.

Another experiment, conducted in 1929, affords further evidence of a longer incubation period for this culture. On March 30 a group of 38 varieties and selections were inoculated with a culture of physiologic form 3 and a similar group with culture 199. The varieties comprised 31 selections all highly resistant to physiologic form 9, 4 selections susceptible to that form and the Australian varieties, Clarendon, Warren, and Thew. Form 3 produced heavy infection on 30 of the selections, a type-2 infection on five selections, and none on three. Culture 199 seemed less virulent, but produced a type-3 infection on 20 selections, a type-2 on eight, a 0 or 0–1-type on five, and an x-type on two. All plants of two other selections died and were discarded. It readily can be seen that, although culture 199 was not quite so virulent as physiologic form 3, the average reaction of the group of varieties to the two cultures was about the same, there being approximately the same number of varieties susceptible and resistant to each culture of rust. The notes on the incubation periods showed wide difference in the time required for the production of visible evidence of flecking and sporulation (Table 5).

TABLE 5.—*Comparison of the average incubation periods of culture 199 and physiologic form 3 on seedlings of 38 varieties of wheat in the greenhouse, 1929*

Total number of selections	Physiologic form 3		Culture 199	
	Number of days from inoculation to			
	Flecking	Sporulation	Flecking	Sporulation
38	5	8	7.4	13.0

In this case flecking was noted for physiologic form 3, an average of nearly two and a half days earlier than for culture 199, while the beginning of sporulation was 5 days earlier.

It already has been stated that all other cultures of *Puccinia triticina* which have been tested in the greenhouse at Manhattan have been characterized by about equally long incubation periods. More than 200 cultures have been studied in detail during the period, 1926 to 1929, inclusive.

Many proved to belong to physiologic forms already described, while others seemed to be new or undescribed forms. Among all these cultures the only one with a strikingly long incubation period was culture 199. All others had developmental periods very similar to that of physiologic form 9. They also all had the same orange red color and large uredinia on susceptible varieties. Culture 199 was not compared with all cultures under study, but was compared with several cultures selected at random both in 1928 and 1929. Inoculations were made with several cultures at the same time and notes taken on date of flecking and sporulation. Data from one such experiment have been presented in table 4. The results of similar experiments conducted in 1928 and 1929 are presented in table 6. It is

TABLE 6.—*Comparison of incubation period and color of uredinia of several cultures of Puccinia triticina on Harvest Queen wheat in the greenhouse 1928 and 1929*

Culture No.	Physiologic form	Number of days from inoculation to			Color of uredinia
		Flecking	Sporulation	Full development	
1928					
199		10	14	20	light orange
119–3	5	5	8	12	orange red
306	9	5	8	12	" "
307	9	5	8	12	" "
198–1		6	8	12	" "
154–4–1	5	6	8	12	" "
170–3		5	8	12	" "
1929					
199		7	14	19	light orange
stock	9	4	7	10	orange red
121–2–1–3	3	4	7	10	" "
119–3–5	5	4	7	10	" "
389–4		4	7	10	" "
157–2–A		4	7	10	" "
362–4		4	7	10	" "

apparent that culture 199 is distinctive from all others, both in length of the incubation period and in spore color as reflected in color of the uredinia.

The differences noted above were sufficiently striking to raise the question whether also there might not be differences in spore dimensions. A number of mounts of spores of form 9 and culture 199 therefore were made and studied. In general characteristics the spores of culture 199 seemed very similar to those of form 9. Their walls were seemingly slightly thinner and therefore somewhat more irregular in shape than those of the spores of

form 9. The lighter color of the spores was not so evident under the microscope as in the spore masses of the uredinia. In spore dimensions there seemed to be a slight difference between the two cultures, although the number of measurements was not large enough to give positive results. Fifty spores each of culture 199 and form 9 were measured for length and width. Although spores of leaf rust are nearly globular they usually are slightly larger in one dimension than another, or have irregularities that affect the spore shape. The results of these measurements gave the following average dimensions in microns:

	Length	Width
Form 9	26.87 ± .159	25.02 ± .134
Culture 199	23.48 ± .140	22.71 ± .125
Differences	3.39 ± .211	2.31 ± .183

These figures seem to indicate that the spores of culture 199 are definitely smaller than those of physiologic form 9, and that the differences are statistically significant. In both length and width measurements the probable error of the differences is less than ten per cent of the difference. The number of measurements is too small, however, to give entirely reliable results.

To determine whether culture 199 differs from known physiologic forms in its behavior on varieties of wheat having differential reactions, or is similar to some known form, several experiments were conducted in 1928 and 1929. It was found extremely difficult, however, to obtain accurate rust readings on differential varieties because of the extremely long developmental period. For all other cultures of rust under investigation readings could be made 12 to 14 days after inoculations. Readings could seldom be made on culture 199 until the 18th day and frequently not until 20 days after inoculation. Seedlings of differential varieties of wheat, inoculated when 10 days old, were 30 days old by that time. The prophylla of seedlings of this age usually have turned yellow and frequently are extremely etiolated. As a result, the plants usually had passed the stage where satisfactory rust readings could be made before culture 199 was fully developed. Very little can be determined about necrosis and flecking due to rust on chlorotic leaves. Many sets of differentials were in such poor condition when the rust had finally developed as to make accurate readings impossible and they were therefore discarded. In a few cases, however, rust readings were made. For reasons given, the results were somewhat variable.

In fact, no completely satisfactory readings have yet been secured on this culture. The most satisfactory readings give reactions somewhat similar to those for physiologic form 10. The comparative infection-type readings for that form and for culture 199 are as follows:

Differential variety	C. I. No.	Infection type	
		Physiologic form 10	Culture No. 199
Malakof	4898	4	3–4
Norka	4377	4	3–4
Mediterranean	3332	1–2	2– to 2
Democrat	3384	1–2	2–3
Hussar	4843	1–2	2–3
Webster	3780	4	3–4
(Unnamed)	3747	3–4	3
"	3756	4	2
"	3778	4	1–2
"	3779	4	3

The differential reaction of the two rusts on Democrat, Hussar, C. I. 3756, and C. I. 3778 makes it seem certain that culture 199 is not physiologic form 10, and, as it resembles no other described form, it probably is a new form.

It should be stated here that no straight type-4 infection has been noted for culture 199. As shown above, a variety occasionally exhibited a susceptibility classified as 3–4, but the copious production of large normal uredinia characteristic of type 4 was not seen. The uredinia always were smaller and less abundant for culture 199. The small size and light color of the uredinia probably had considerable effect on the infection types assigned a variety in making rust readings. Thus it appears that culture 199 is a different physiologic form, although it somewhat resembles physiologic form 10. Aside from the differential reaction noted above, the long incubation period, light-color spores, and small uredinia of culture 199, also, would place it in a separate category.

CONCLUSIONS

When all characteristics of culture 199 are considered, the possibility of its origin through mutation is strongly suggested. There is, however, no direct evidence in proof of the point. Even circumstantial evidence is entirely too meager to justify such a conclusion. The mutations noted by Newton and Johnson[8] occurred under observation and therefore cannot be

[8] *Loc. cit.*

questioned. The origin of culture 199, on the other hand, is entirely unknown.

It is certain, however, that culture 199 is an aberrant type of *P. triticina*. It differs distinctly from all other cultures yet studied in the greenhouse at Manhattan, Kansas, in length of incubation period, spore color, and size of uredinia on susceptible varieties. Those characters also were evident in the culture when collected in the field. It was noticed and collected because it appeared very late in the season on a highly resistant variety and was characterized by a small number of minute, pale uredinia. Regardless of the origin the culture is interesting in the constancy with which the variations noted above were expressed in repeated transfers. The writer has frequently noted a sparse infection of leaf rust on highly resistant varieties in the field late in the season. Such infections usually were characterized by minute uredinia, frequently of very light color. These usually were regarded as late-developing infections due to the resistance of the host. Such actually may have been the case, but, on the other hand, they may have been due to the presence of an aberrant type of leaf rust similar to that of culture 199.

SUMMARY

An aberrant form of leaf rust of wheat, *Puccinia triticina*, was found on a highly resistant strain of Mediterranean wheat, Texas 3015–63, at Denton, Texas, in the spring of 1927. This culture is discussed as culture 199.

Greenhouse experiments have shown that this form differs from all other cultures studied at Manhattan, Kansas, in length of incubation period, color of spores, size of uredinia, and spore dimensions.

The period from inoculation to flecking averages about three days longer, and that from inoculation to sporulation about seven days longer, than for other known forms. The time required to reach full development also is much longer for culture 199.

The spores are a lighter orange in color than those of other forms and the uredinia are smaller. Spore measurements indicated that spores of culture 199 were slightly smaller than those of form 9.

It was proved that the peculiar behavior of culture 199 was not due to the resistance of the host variety.

On differential varieties the culture gave reactions indicating that it was an undescribed physiologic form. The aberrant nature of the culture, however, makes its identity doubtful.

The aberrant type may have arisen through mutation, but only meager evidence is available on that point.

THE RELATION OF TYPE OF TOPPING TO STORAGE LOSSES IN SUGAR BEETS[1]

C. M. TOMPKINS AND S. B. NUCKOLS[2]

INTRODUCTION

In an examination of stored beets at Lewiston, Utah, on December 20, 1927, after they had passed through the washer in the factory mill, it was noted that storage rots were doing considerable damage. Fully 50 per cent of the beets in the hopper showed more or less decay, the amount varying from small involvements of crown to total decay of the root. The commonest type of rotting in this stored material was what is called in this paper "crown rot" to distinguish it from the other decays of beets that arise from wounds or bruises or that may arise from the drying of the small side roots or the tapering tip of the main root. This distinction is made, although probably the same decay-producing organisms are involved in the rotting, because of the clear-cut relation of the crown-rot type to the invasion of the crown surface of the beet exposed by topping. In the observations made at Lewiston, Utah, and which were later repeated at Logan, Utah, beets with the greater portion of the crown removed by topping showed the most rotting. Those beets on which a large portion of the crown remained were almost free from decay, while beets topped at the line of the lowest leaf scar (the approved commercial method of topping) showed approximately 50 per cent in various stages of decay. Those beets topped one-fourth inch or more below the base of the lowest leaf scar were in nearly all instances severely attacked by decay organisms.

These observations led the writers to begin investigations upon the nature of crown rot of stored beets, its seriousness, and the connection of the rot to the type of topping practiced. The investigations undertaken have involved some isolation work along with inoculations to indicate the organisms concerned with decay of sugar beets in storage piles, and a thorough examination of the beets in the storage pile to determine what relations existed between crown rot and the type of topping which had been given the individual beets.

[1] The writers are indebted to Dr. B. L. Richards, Utah Agricultural Experiment Station, and Dr. G. H. Coons, Office of Sugar Plants, U. S. Department of Agriculture, for kindly suggestions and critical reading of the manuscript. Appreciation is also expressed to The Amalgamated Sugar Company, Ogden, Utah, in whose laboratories all chemical analyses were made.

[2] Assistant Pathologist and Associate Agronomist, respectively, Office of Sugar Plants, U. S. Department of Agriculture.

THE ORGANISMS CAUSING CROWN ROT

From among 10,945 beets examined at Logan, Utah, 400 sugar beets showing definite symptoms of the crown rot described were selected for isolation purposes. Two small fragments from margins of lesions were cut from each beet under aseptic conditions and planted on potato-dextrose agar to which had been added several drops of 10 per cent lactic acid to prevent bacterial contamination. Strong fungous growth was obtained from nearly all within 36 hours. Transfers were made to tubes of potato-dextrose agar by picking off hyphal tips at the edge of the colonies by means of a sterile platinum needle. The cultures in the tubes were incubated for one week to insure sporulation, after which cultures were made from single spores by plating. From the 800 tissue plantings, 381 cultures of *Phoma betae* and 339 isolations of two species of Fusarium (*Fusarium culmorum* and *Fusarium* sp., similar to *F. oxysporum*), were obtained, the remaining 80 tissue plantings failing to give fungous growth.

This large number of tissue plantings was made in order to determine the relative frequency of *Phoma betae* and the two species of Fusarium in diseased tissue. This was suggested as a result of isolations made from a limited amount of material collected at Lewiston, Utah, as the beets were dropping into the hoppers in the factory immediately following washing. Isolations from the Lewiston beets yielded *P. betae* mainly, but two different species of Fusarium were also frequently obtained. The results of isolations made from rotted sugar beets collected at Lewiston and Logan, Utah, indicate that *P. betae* is extremely important as a septic factor in sugar beets, since it was associated in more than one-half of the cases of crown rot from which isolations were made. Two species of Fusarium are also concerned in this type of decay. These have been classified as follows by Dr. C. D. Sherbakoff.[3]

> "Isolations 258 and 263: *Fusarium culmorum;* the strain with commonly shorter, 3-septate conidia as in *F. discolor* var. *triseptatum* Sherb. The last named fungus appears to be identical with this strain of *F. culmorum.*" (Fig. 1, *1* and *2.*)
>
> "Isolations 262 and 264: *Fusarium* sp.; in microconidia, is similar to *F. oxysporum;* macroconidia few and poorly developed or none (hence the difficulty of identification); on agars with glucose, color often purplish slate; chlamydospores and other characters of section Elegans." (Fig. 1, *3* and *4.*)

[3] The writers wish to express their appreciation to Dr. C. D. Sherbakoff, Plant Pathologist, Tennessee Agricultural Experiment Station, for study given the various isolations of Fusarium sent him and for his suggestions as to the names provisionally to be assigned to these organisms.

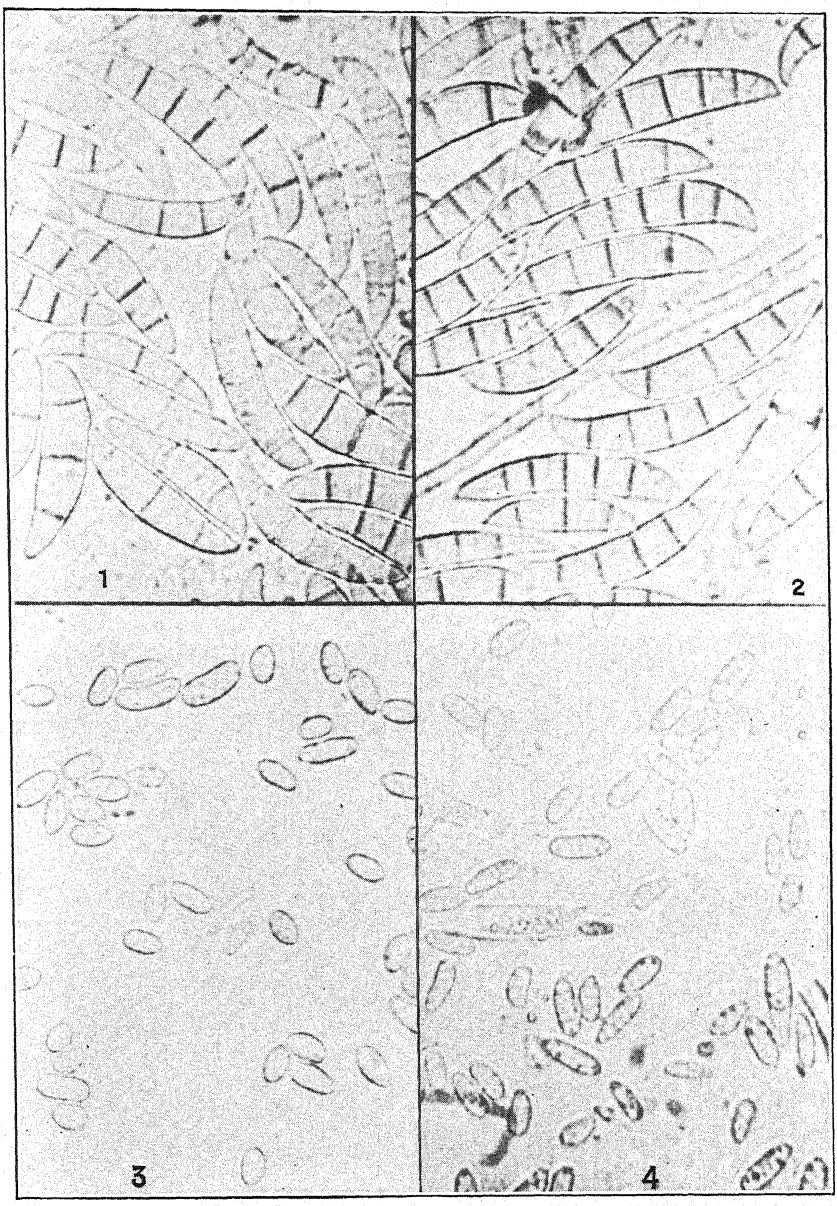


FIG. 1. *1* Macrospores from *Fusarium culmorum,* Isolation 258; *2* Macrospores from *F. culmorum,* Isolation 263; *3* Microspores from *Fusarium* sp. from Isolation 262; *4* Microspores from *Fusarium* sp., Isolation 264 (×1000). Photomicrographs through courtesy of Dr. C. D. Sherbakoff.

TABLE 1.—*Inoculations of healthy roots of sugar beets with Phoma betae and Fusarium* sp. (*Inoculated February 15, 1928; read March 31, 1928*)

Organism	Isolation No.	Source of organism	No. of beets inoculated	No. of beets diseased in 45 days	Percentage diseased in 45 days	Controls	
						No. of beets used	No. of beets healthy at close of 45-day period
Phoma betae	259	Logan, Utah	70	56	80.0	10	10
" "	260	" "	70	60	85.7	10	10
" "	261	" "	70	70	100.0	10	10
" "	265	Lewiston, "	70	70	100.0	10	10
Fusarium culmorum	258	Logan, "	70	57	81.4	10	10
	and 263	Lewiston, "	70	55	78.5	10	10
Fusarium sp. (Similar to *Fusarium oxysporum*)	262 and 264	Lewiston, "	70	40	57.1	10	10
		" "	70	16	22.8	10	10

For inoculation purposes a large number of healthy sugar beets were treated ten minutes in a solution of 1 to 1000 mercuric chloride solution and then washed successively in tap, distilled, and sterile water. With a large, stainless steel knife, previously treated by dipping in 95 per cent alcohol and flaming, a thin slice of tissue was cut from each beet parallel to the original topped area, thus exposing fresh tissue free from cork cells. Week-old cultures of *Phoma betae* (represented by four isolations) and of the two species of Fusarium (represented by two isolations of each species) on prune agar were used for the inoculations. The inoculum, consisting of a block of agar approximately 2 cm. square by 0.5 cm. deep, plus growth, was placed on the freshly exposed cut surface, with the fungus in direct contact with the beet tissue. A piece of sterile cotton moistened with sterile water was then placed over the inoculum and held in position by means of a strip of adhesive tape. The beets, after inoculation, were placed in glassine bags and stored in a cellar where the air temperature was approximately 4° to 6° C. Results of inoculations are given in table 1. Reisolations were made in each case from beets in which decay had been induced artificially and the fungus again obtained in pure culture.

From this table it is clear that rotting of stored sugar beets can be inaugurated in a high percentage of cases by *P. betae* and the two Fusarium species tested, if these fungi are introduced in considerable mass upon freshly wounded crown tissue. It can not be said that the variation in percentages of disease production is indicative of the aggressiveness of the particular pathogenes, since variations in the reaction of the host, in storage conditions, and other factors may have influenced the results. We may, however, from these inoculations, definitely assign to certain pathogenes a causal connection with the decay of sugar beets occurring in storage piles. Probably other fungi are occasionally concerned in this decay, and many species of fungi and bacteria invade the attacked tissues as secondary invaders.

THE RELATION OF CROWN ROT TO THE TYPE OF TOPPING

Sugar beets which had been commercially topped, stored at Logan, Utah, in dirt pits, and covered with a thin layer of moist sand were available for rather complete study. These beets were removed from the pits after being in storage 84 days. The conditions for storage were presumably as good as, if not better than, would be found in commercial storage. It is not assumed that the same amount of rot would be found in all commercial storage piles, since the period was greater than the ordinary period of storage. It is probable, however, that the disease conditions prevalent were approximately the same as with similar beets in commercial storage piles where storage rots were serious.

In examining these beets considerable variation was found in the amount of crown taken off in the topping process. The reason for this is apparent when the methods used in the field are considered. In handling the beets for topping with a hooked beet knife, the laborer lifts the beet from the ground to his left hand by use of the hook. One quick stroke usually severs the crown from the root at approximately a right angle to the longitudinal axis of the beet. The instructions are to make this cut at the base of the lowest leaf scar, but unavoidable variations enter. The laborer usually does not attempt to correct imperfections in topping, with the result that beets are delivered to the factory with the majority approximately correctly topped, while the others are topped above or below the lowest leaf scar.

In order to make a careful study of stored beets and the relation of topping to the amount of fungous decay, five arbitrary divisions of the types of topping were made. The beets in the storage pit used for this investigation were examined and placed in the class which most nearly represented its individual type of topping. In cases where the cut was slanting instead of at a right angle to the longitudinal axis, the beets were assigned to the class represented by the lowest angle of the cut.

For convenience, these classes are designated:

Class a—To include all beets topped one-half inch or more above the lowest leaf scar, leaving a large amount of crown attached to the root.

Class b—To include all beets topped approximately one-fourth inch above the lowest leaf scar, leaving a smaller amount of crown attached to the root.

Class c—To include all beets topped on a line at the base of the lowest leaf scar. This would remove all of the leaf scars from the beet, leaving all of the root tissues intact with no crown adhering.

Class d—To include all beets topped one-fourth inch below the lowest leaf scar. This would remove approximately one-fourth inch of the root with the crown.

Class e—To include all beets topped one-half inch below the lowest leaf scar. This would remove one-half inch or more of the root with the crown. (Fig. 2, A.)

Class *c* represents the standard commercial topping. Commercial companies do not object to beets topped as represented by Classes *c, d,* and *e,* although they recognize as wasteful the topping described in Classes *d* and *e.* The companies do object to topping as represented by Classes *a* and *b,* and, through tare sample determinations, they arrive at the extra crown weight of such beets and deduct the same from the gross weight of beets delivered.

In this study all beets were classified as diseased or healthy according to the presence or absence of rotted areas on the cut surface. Beets showing a healthy condition of this cut surface, but having decayed areas in

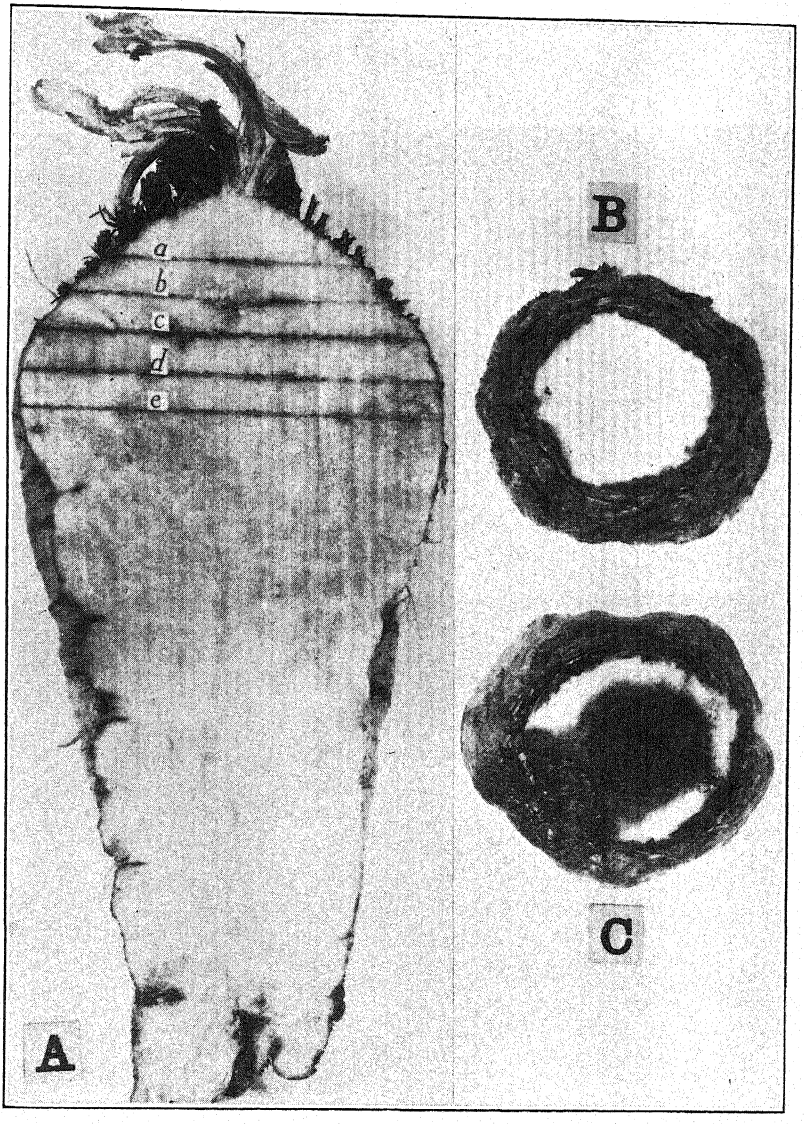


FIG. 2. A, Chart adopted for classification of sugar beets according to type of topping. Line *c* marks the point of the lowest leaf scar which is the point of commercial topping. B, Healthy and C, diseased crowns of sugar beets topped at *a*. Storage period 84 days.

other portions of the root arising from other injuries, were classed as healthy because their decay bore no relation to injury induced by removal of the crown.

The beets were removed from this pit and were classified according to the five types of topping detailed above. The presence or absence of decayed areas on the cut surface of the root was then noted. Beets free from decay in the crown portion of the root were classed as healthy. Beets with one or more decayed areas on the cut surface were classed as diseased. There was considerable variation in the amount of decay on individual beets, some having very small decayed areas and others showing badly rotted areas.

The Class *c* type of topping is the approved commercial method of topping, and the greatest number of beets examined were found to be topped at the line *c* (Fig. 2, A). More than 50 per cent of the beets were properly topped and there seemed more tendency to leave a part of the crown on the beet than to top the beet too low. The results of this classification of the beets and the relative amount of crown rot in the various classes are shown in table 2. The definite and consistent trend in percentages of rotted and sound beets in the various established classes indicates that the type of topping has a direct effect upon the incidence of infection by parasitic fungi in stored beets. The beets in Class *c*, conforming to the approved commercial type of topping, were found to be 37 per cent healthy and 63 per cent diseased at the end of the 84-day storage period. Lower topping increased the amount of decay so that approximately four-fifths of the beets of Classes *d* and *e* showed various degrees of crown rot. Approximately two-thirds of the beets topped at the line *b* were healthy, while in those topped at the line *a* there was approximately only one diseased beet in seven. These strongly contrasting figures indicate close relation of type of topping to the numbers of individuals showing crown rot in storage, and that there is a marked increase in rotting as the plane of topping is lowered.

TABLE 2.—*Relation of type of topping to the keeping qualities of stored sugar beets: Commercial beets from Logan, Utah, stored in covered pits for 84 days*

Type of topping	Total		Number of beets		Percentage	
	No. beets	Percentage	Healthy	Diseased	Healthy	Diseased
a	730	6.67	632	98	86.58	13.42
b	2933	26.80	1999	934	68.16	31.84
c	5616	51.31	2085	3531	37.13	62.87
d	1086	9.92	198	888	18.23	81.77
e	580	5.30	118	462	20.34	79.66
Totals ...	10945	100.00	5032	5913	45.98	54.02

There also appeared to be marked variation in the degree of involvement by crown rot in the various classes of beets. Table 3 gives the results of determinations made upon 90 beets representative of the rotted beets of the five classes. The actual amounts of sound and diseased tissue were determined for these samples and the percentage of involvement computed. These percentages are believed to be fair indexes of the conditions of the rotted beets in the various classes (Figs. 2 and 3). On this basis, as can be seen from the table, the rotted beets of Class *c* had approximately 14 per cent of their tissue affected at the end of the storage period. The tissue involvement increased with lower topping, rotted beets from Classes *d* and *e* showing, respectively, 4 and 8 per cent more of their tissue decayed than was found in Class *c* beets. On the other hand, Classes *a* and *b* beets

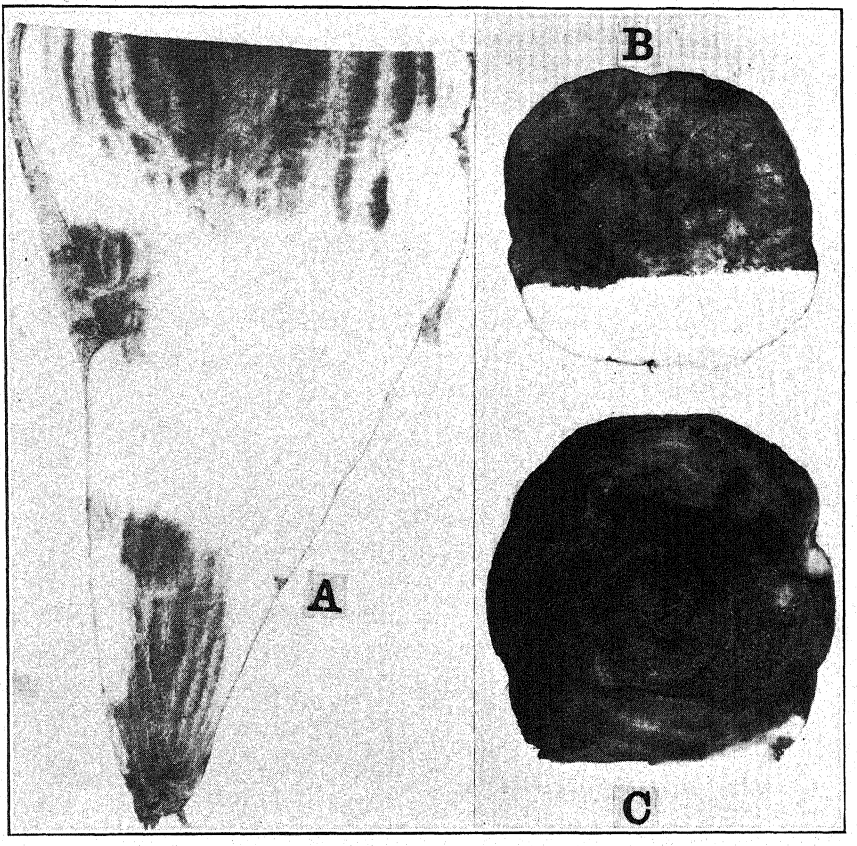


FIG. 3. A, Longitudinal section of sugar beet topped at *d*, showing advanced stage of decay. B, Healthy, C, diseased crowns of sugar beets topped at *e*. Storage period 84 days.

showed, respectively, 8 and 6 per cent *less* tissue involvement than Class *c* beets.

The percentages of involvement in the rotted beets, shown in table 3, when considered in connection with the actual numbers of sound and rotted beets of the respective classes, as shown in table 2, reveal a very significant situation as to total weight of beet tissue in the various classes affected by rot.

TABLE 3.—*Relation of type of topping to the extent of diseased tissue in stored sugar beets. Storage period 84 days. The beets used in this test showed distinctly that the origin of infection was from the crown scar*

Type of topping	Number of beets examined	Gross weight of beets (grams)	Weight of healthy tissue (grams)	Weight of diseased tissue (grams)	Percentage of total tissue diseased
a	8	6124	5835	289	4.72
b	16	13695	12553	1142	8.35
c	21	18976	16189	2787	14.69
d	18	14116	11816	2300	16.29
e	27	17669	13626	4043	22.88
Totals	90	70580	60019	10561	Average 14.96

Class *c* beets came out of storage with approximately 63 per cent of the beets affected with crown rot. Approximately 15 per cent of the total weight was decayed. In this case, there would be a loss of nearly 10 per cent of the total weight of *c* beets due to crown rot in storage. The shrinkage due to rot is still greater for Class *d* and Class *e* beets, being approximately 14 and 17 per cent of the respective total weights, which shows that topping at the line *d* or *e* has grave disadvantages in addition to the regular topping loss which comes in the field. Class *b* beets had approximately 32 per cent of the individuals diseased and an average of 8.3 per cent of the tissue damaged. This is equivalent to shrinkage from rot of 2.66 per cent of the total weight of all beets of this class. In Class *a* 13.4 per cent of the individuals were diseased, and these showed 4.7 per cent of the tissue decayed. This indicates that Class *a* beets sustained a loss of only 0.63 per cent of their total weight from crown rot during the 84-day storage period.

From common knowledge of the nutritional requirements of the fungi and bacteria which cause decay of beets, it is safe to assume that in the processes of decay the sugar of the involved tissue would be destroyed by the attacking organisms. The accuracy of this assumption is borne out by the analyses given in table 4 in which composite samples of many beets, but with varying percentage of rotted tissue, were used.

The samples were made up of beets showing approximately the same ratio of diseased and healthy tissue as was found in the general run of beets in the different classes from the storage pit. It is noticeable that Class *a* beets, which, because of their high tops, probably had the lowest amount of total sugar, came through the storage period with the highest sugar content and highest purity. It will be noted from the table that the sugar percentage of the beets dropped in close proportion to the percentage of rot and that the purities were strongly affected. The results shown in this table give some indication of how important losses of this type may become.

TABLE 4.—*Post-storage analyses of differently topped sugar beets. Storage period 84 days*

Type of topping	Healthy tissue in sample	Diseased tissue in sample	Sugar in beets	Purity of juice
	Per cent	Per cent	Per cent	Per cent
a	99.35	0.65	18.09	84.3
b	97.33	2.67	17.96	82.7
c	90.77	9.23	17.13	80.6
d	86.92	13.08	15.37	73.8
e	81.77	18.23	14.51	70.1

It is obvious from the data presented that from the standpoint of crown-rot prevention, the practice of leaving a higher crown in topping must be considered. Offsetting the savings which come from prevention of loss of extractible sugar by decay, there must be taken into account the general effects on sugar and purity which arise from the well-known lower sugar and high mineral content of the sugar-beet crown. Factory operators object to any practice which tends to lower sugar content or purity of juice in the sliced product because these items tend to increase manufacturing difficulties and cost of production.

In the beets studied in these experiments there were no indications of sprouting of the stored beets. The present paper offers no data on the effect of greater crown portions on the sprouting of beets stored under conditions favorable to the growth of sprouts on the beets. In case sprouting were brought about by storage conditions, there would presumably be the greatest growth of sprouts on those beets having the greatest amount of crown, and this also would offset to some degree the savings indicated by the crown-rot prevention. In average seasons, however, air temperatures during the storage period are generally low enough to preclude the growth of sprouts.

Since work on this problem did not commence until the end of the factory campaigns in the Western States, no harvest data are available on the percentage of sugar, purity, and ash analyses of beets corresponding to the classes set up in this paper. As a substitute for freshly harvested beets, some analyses were made of beets kept in cold storage 84 days. These beets had been held at a temperature which precluded infection and were in a crisp condition and free from all signs of decay. When harvested the entire crown was left on the beet root. Although beets undergo some chemical changes in storage, it is believed that the relations in the various portions in these beets approximate those of freshly harvested beets. These whole beets were sectioned so as to obtain material representing the portion of the beet which would be removed by successive topping at the five arbitrary points *a*, *b*, *c*, *d*, and *e*. The portion removed by topping at the line *a* contained relatively the lowest sugar content and the highest ash content. The portions removed by lower topping had a successive increase in sugar content and a successive decrease in ash content (Table 5). These data correspond closely with data published by other authors who worked with fresh beets.

TABLE 5.—*Post-storage analyses of sections of the crown and root of healthy sugar beets. Analyses of 38 beets kept in cold storage 84 days. Percentage of ash based upon weight of dry substance*

Section of beet	Sugar	Soluble ash	Insoluble ash	Total ash
	Per cent	Per cent	Per cent	Per cent
Above *a*	8.4	5.60	10.51	16.11
Between *a* and *b*	11.6	4.17	5.92	10.09
Between *b* and *c*	14.9	2.67	2.74	5.41
Between *c* and *d*	15.1	3.03	1.58	4.61
Between *d* and *e*	16.0	2.64	1.50	4.14
Below *e*	16.7	2.55	1.45	4.00

By use of the data obtained from analyses of various sections of the beet and by determination of the weight of the various sections, a calculation was made to obtain information regarding the weight, sugar content, and ash content of the remaining root that would be marketed by the different types of topping. This indicates that topping above the line *c* would increase the marketable weight of sugar beets and that such beets would have a slightly higher ash content and a slightly lower sugar content than

beets topped at the line *c* (Table 6), but the depreciation of sugar-beet quality which would arise from a change in practice to permit higher topping if either line *a* or line *b* were taken as a standard seems to be far less than the loss possible in storage piles where crown rot is serious.

TABLE 6.—*Comparison of weight, sugar, and ash content of differently topped sugar beets. Stored 84 days. Percentages computed in terms of beets topped in standard manner (Class c)*

Portion of beets (38 beet sample)	Total weight (ounces)	Weight	Sugar	Soluble ash	Insoluble ash	Total ash
		Per cent	Per cent	Per cent	Per cent	Per cent
Whole beets	1019	126.1	15.54	2.87	2.44	5.31
Root below line *a*	951	117.7	16.05	2.71	1.89	4.60
b	888	109.9	16.36	2.60	1.58	4.18
c	808	100.0	16.51	2.59	1.47	4.06
d	742	91.8	16.63	2.56	1.46	4.02
e	670	82.9	16.70	2.55	1.45	4.00

The present standards as to removal of crowns from beets have been based upon consideration of the condition of the beets at harvest time. The above tables indicate that under conditions where crown rot is prevalent during the storage period there is a benefit to be derived from leaving a part of the crown on the root. Since higher topping inhibits the development of crown rot to a marked degree in stored beets, sugar companies should consider carefully the savings possible by the practice of topping beets intended for storage higher than the present standard. The change in method of topping should not be made by any factory without a thorough study of local conditions as to seriousness of crown rot and as to effect of a change of practice on the quality of the beets. If crown rot is as serious as the writers' observations indicate, factories may find it to their advantage to secure beets that have been so topped as to leave part of the crown on the root.

DISCUSSION

At present, sugar beets are topped commercially at the base of the lowest leaf scar, in preparation for sugar extraction at the factory, regardless of whether they are to be used immediately or stored from 25 to 40 days or longer. Results of preliminary observations made at Lewiston, Utah,

and experimental work conducted in the field and laboratory at Logan, Utah, appear strongly to support the statement that, where sugar beets intended for storage purposes are topped at the base of the lowest leaf scar or lower, more beets show crown rot and the rot involves more tissue than when the beets are topped one-quarter to one-half inch above this region. In Cache Valley, Utah, more than 100,000 tons of beets were stored for an average period of 40 days. Experimental data obtained at Logan indicate that there may be from 10 to 15 per cent shrinkage of the beet substance suitable for sugar extraction from this one source, alone, where storage conditions are such that rot progresses strongly. It is believed that up to a certain degree, losses of the same character may occur fairly generally in commercial beet storage piles in the Western States. Since no survey has been made to establish loss figures other than in Cache Valley where 10 per cent of the crowns showed some degree of rot, one can only surmise what the actual loss may be in a season favoring storage rotting. Experimental consideration of this method of topping beets intended for storage by beet-sugar manufacturing companies may lead to changes in the present methods of topping of beets for storage and a partial saving of the present losses of sugar from stored beets.

Based on data collected in this study, approximately 7 per cent of the total number of beets milled are topped at *a*, or one-half inch above the base of the lowest leaf scar, and 27 per cent are topped at *b*, or one-quarter inch above the *c* region. Factory operations have not been impeded by the use of 34 per cent of the total number of beets milled which are topped at *a* and *b*. If all beets intended for storage were topped at *a* or even at *b* the amount of decayed tissue in the crowns which develops during storage would be decreased, while, on the other hand, there would be a greater tonnage for milling than where beets are topped at the line *c* or lower. From the data obtained, it is believed that, for stored beets where rotting is serious, the prevention of loss from rot which would come from leaving a higher crown on the beet would far more than offset the depreciation in quality.

Several theories have been considered as an explanation for the variation in the amount of decay in the crowns of stored beets. Morphologically, considerable difference is found in beet tissues in the five regions adopted for classification. In regions *a* and *b*, the fibrovascular bundles or rings are closer together and the amount of storage parenchyma is reduced. In the regions *c*, *d*, and *e*, parenchymatous tissues predominate, while the vascular rings are widely separated. Chemical analyses indicate a slight difference in percentage of sugar, purity, and ash content for the several regions. The organisms concerned in sugar-beet-crown decay are apparently better able to penetrate into spongy tissues where there is a high percentage of sugar (as in the case of beets topped at *c*, *d*, and *e*) than they

are in regions such as *a* and *b* where the spongy parenchyma is reduced, the sugar content is lower and the ash content is higher.

Undoubtedly studies on the rate of formation and the relative thickness of the corky layer formed on the cut surfaces of beets topped at different planes will prove to be important as means contributing to the final solution of the problem. Studies on these phases of the problem are now in progress.

SUMMARY

Isolation and inoculation studies indicate that *Phoma betae* is the most important pathogene concerned in the destruction of crown tissues of sugar beets under storage conditions, although two species of Fusarium are pathogenic to the sugar beet and may be of economic importance. All are wound parasites, but *P. betae* destroys crown tissues with greatest rapidity.

This paper constitutes a preliminary report on the correlation of storage losses in sugar beets with types of topping.

More than 10,000 beets were examined and classified as healthy or diseased. All beets were further classified according to the five divisions adopted for the description of the types of topping of commercial sugar beets.

Approximately 51 per cent of the total number of beets examined were topped at the base of the lowest leaf scar (*c*), or the commercial standard, 37 per cent of which were healthy and 63 per cent diseased. A marked decrease in numbers of diseased beets resulted when beets were topped one-fourth inch (*b*) and one-half inch (*a*) above this line, while a very decided increase in numbers of diseased beets occurred when the crowns were severed one-fourth inch (*d*) and one-half inch (*e*) below this line.

Where sugar beets were topped at the base of the lowest leaf scar (*c*) and lower (*d* and *e*), the amount of tissue which was decayed varied from 14 to 25 per cent; above this region (*a* and *b*) the amount of tissue which was decayed varied from 4 to 8 per cent.

Increased avoidance of attack by fungi was shown by stored beets topped higher on the crown than the topping practice recommended by commercial companies.

It is believed that sugar-beet companies could avoid a considerable part of the heavy loss from crown rot of stored beets by revision of the commercial standards for topping of the beets intended for storage.

OFFICE OF SUGAR PLANTS,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
IN COOPERATION WITH
UTAH AGRICULTURAL EXPERIMENT STATION,
LOGAN, UTAH.

INFECTION PHENOMENA AND HOST REACTIONS CAUSED BY TILLETIA TRITICI IN SUSCEPTIBLE AND NONSUSCEPTIBLE VARIETIES OF WHEAT[1]

H. M. WOOLMAN[2]

INTRODUCTION[3]

The investigation of the wheat-bunt problem of the Pacific Northwest, carried on by the Office of Cereal Crops and Diseases, in cooperation with the State experiment stations of Oregon and Washington, has shown conclusively that it can be solved most satisfactorily by the production of bunt-immune or highly resistant varieties. To eradicate bunt by this means, it is evident that varieties must be produced in which are combined the qualities of immunity from the bunt organisms, *Tilletia tritici* (Bjerk.) Wint. and *T. laevis* Kühn, and such other desirable characters as improved yield, strength of straw, and good milling and baking qualities. In other words, they must be at least as profitable when grown as are the susceptible varieties now in cultivation. In undertaking such a difficult problem, it seemed desirable first to have the fullest possible knowledge regarding all factors thought to influence resistance.

Observations made during several years' testing of varieties for bunt resistance have shown that the plants of many partially resistant varieties are able to suppress to a certain degree further invasion of their tissues by the fungus after infection takes place. This was indicated by the presence of both bunted and normal heads in stools of single plants at harvest time. The degree of smuttedness in these partially bunted plants varied from one grain of wheat in an otherwise totally bunted plant to the opposite extreme of one bunted grain in an otherwise bunt-free plant.

The purpose of these investigations, begun in 1919, was to determine, as far as possible, the nature of the factors underlying resistance. Are there varieties partially or totally resistant to the initial entrance of the bunt organism? In other words, is resistance due wholly or in part to the

[1] Cooperative investigations conducted by the Oregon Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

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[3] The writer desires to express his obligations to Professor H. P. Barss, of the Oregon Agricultural College, for assistance in interpreting results of cytological slides; and to Dr. W. H. Tisdale, formerly of the Office of Cereal Crops and Diseases, and Dr. H. B. Humphrey, of the Office of Cereal Crops and Diseases, for assistance in preparing this manuscript.

structural nature of the epidermis? Or is it due wholly to the chemical nature of the cell contents? If the latter, at what stage in the development of the host plant do the inhibitive factors come into action?

WORK OF PREVIOUS INVESTIGATORS

So far as the writer has been able to ascertain, there is extant no literature dealing with the difference in reaction of the host to the bunt organism between susceptible and nonsusceptible varieties. As regards the mode of infection by *Tilletia tritici* or *T. laevis,* the development of the fungus within the host, and its physiological relations thereto, previous work is confined to Kühn, William Lang, Dastur, and Bolley. Brefeld and Wolf did not use Tilletia in their smut-infection studies.

The work of Kühn[4] is too well known to need comment, and to this day his conclusions remain unchallenged except, probably, in some particulars, by Lang. Kühn found the fungus hyphae to be partially intracellular and that they readily passed through the cell walls. Lang,[5] however, found them to be entirely intercellular. The writer frequently finds them to be intracellular in the coleoptile and the sheaths of the first and second true leaves and, therefore, in agreement with the observations of Kühn. He also finds that it is always intercellular in the nodes, internodes, and spike and in the blades of very young leaves, thus confirming Lang's observations.

The hyphae are often very difficult to find, owing to the fact stated by both Kühn and Lang that the protoplasm moves forward with the growth of the hypha, leaving a thin-wall, empty tube behind, which stains with difficulty and apparently is finally dissolved or digested by the plant juices and disappears entirely.

Bolley[6] made an extensive investigation of the relation of host to parasite in the mature plant but was led to erroneous conclusions, which fact he was the first to discover. Dastur[7] studied the entrance of the fungus to the epidermal cells and states that it gains entrance generally between the cells which it forces apart but that occasionally it passes directly through the epidermis into the lumina of the cells. He states that he obtained his infections by applying a culture of *T. tritici* spores to plants one day old, incubating them at 50° C. Judging from his figures, this abnormal

[4] Kühn, J. Die Krankheiten der Kulturgewächse, ihre Ursachen und ihre Verhütung. Aufl. 2, pp. 22, 312. 1859. Berlin.

[5] Lang, Wilhelm. Ueber die Beeinflussung der Wirtpflanze durch *Tilletia tritici.* Zeitschr. f. Pflanzenkrankh. 27: 80–99. 1917.

[6] Bolley, H. L. New studies upon the smut of wheat, oats, and barley with a resumé of treatment experiments for the last three years. N. Dak. Agr. Exp. Sta. Bul. 27: 109–164. 1897.

[7] Dastur, Jehangir Fardunji. Cytology of *Tilletia tritici* (Bjerk.) Wint. Ann. Bot. 35: 399–407. 1921.

temperature has produced infection that is morphologically very different from that obtained by the writer, whose cultures were maintained at 15° C., the optimum for the normal growth of the bunt organism.

A preliminary summary[8] of the results obtained by the writer has already been published.

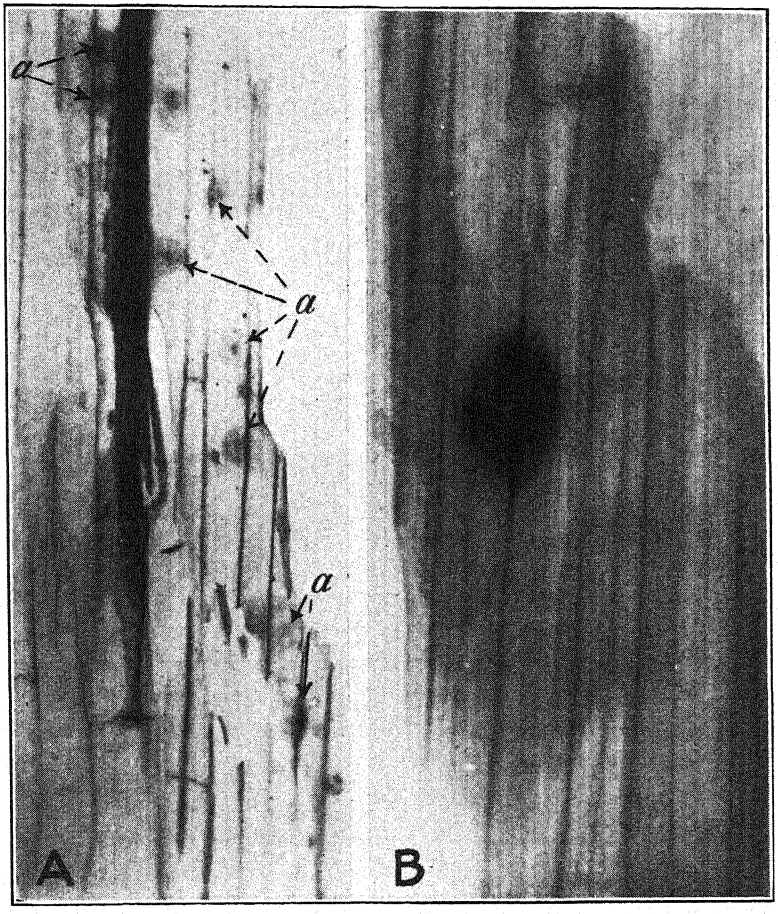


FIG. 1. A. A tangential surface section of the coleoptile from an 8-day plant of Hybrid 143 (susceptible), showing irregular grouping of points of attack, as at *a* (×200). Photograph by Dr. Erwin F. Smith. B. A tangential, near-surface section of the coleoptile from a Martin (nonsusceptible) plant about 7 days old, showing intense gram-positive area around point of entrance (×580). Photograph by Dr. Erwin F. Smith.

[8] Woolman, H. M. Cytological studies on the infection of wheat seedlings by *Tilletia tritici* (Bjerk.) Wint. (Abst.). Phytopath. 13: 36–37. 1923.

Phases of Infection. For convenience, the development of the fungus within the host can be divided into three phases.

First: The entrance into and development within the epidermal layer of cells, during which time it is gram-negative in its staining reactions and both intercellular and intracellular in habit. (Figs. 1–5.)

Second: Its development in the deeper parts of the coleoptile and in the sheaths of the true leaves, especially the first and second leaves, where it is gram-positive and both intercellular and intracellular. (Fig. 6.)

Third: Its development in the very young leaf blades, the nodes, internodes, and the growing point, where it is gram-positive and strictly intercellular. (Fig. 7.)

INVESTIGATIONS

The work in 1919 to 1920, inclusive, was performed under great difficulties, due in part to inadequate equipment and in part to the writer's own shortcomings. The thermostat, supposed to control the hot-air oven in which the embedding was done, frequently failed to control the temperature, and, as it was impossible to keep a day and night watch, several lots of plants were lost from overheating. Others were so overhardened that parts were lost in the process of cutting, fixing, and staining. As a consequence of the foregoing facts and the faulty technique, the work from 1919 to 1920, inclusive, deals only with the second and third phases of infection. The first or entrance phase was not observed.

Method of obtaining infected plants. A mass of steam-sterilized soil was air-dried and mixed with 2 per cent of its weight of crushed bunt balls. This mixture was then divided into four equal parts, each part in succession being well moistened at two-day intervals. When the last part was moistened, all were thoroughly mixed together and the seed planted at a uniform depth of 5.5 cm. At this time, the spores in the portion first moistened had germinated and were producing conidia profusely. Thus the presence of an active and virulent inoculum was insured for several days after planting.

During the entire time from preparation of the infested soil until the last plants were removed for killing, the operations were conducted in a greenhouse maintained at approximately 15° C.

Killing and staining technique. The plants were killed in a mixture of equal parts of absolute alcohol and glacial acetic acid. They were then run through absolute alcohol to xylol and embedded in 60° paraffin.

The fungal hyphae are generally gram-positive in their staining reactions when within the host. Therefore, gentian-violet, followed by Gram's iodine solution, was used as a differential stain. Orange G or eosin was employed as a counter stain. However, at its initial entrance into the epidermal

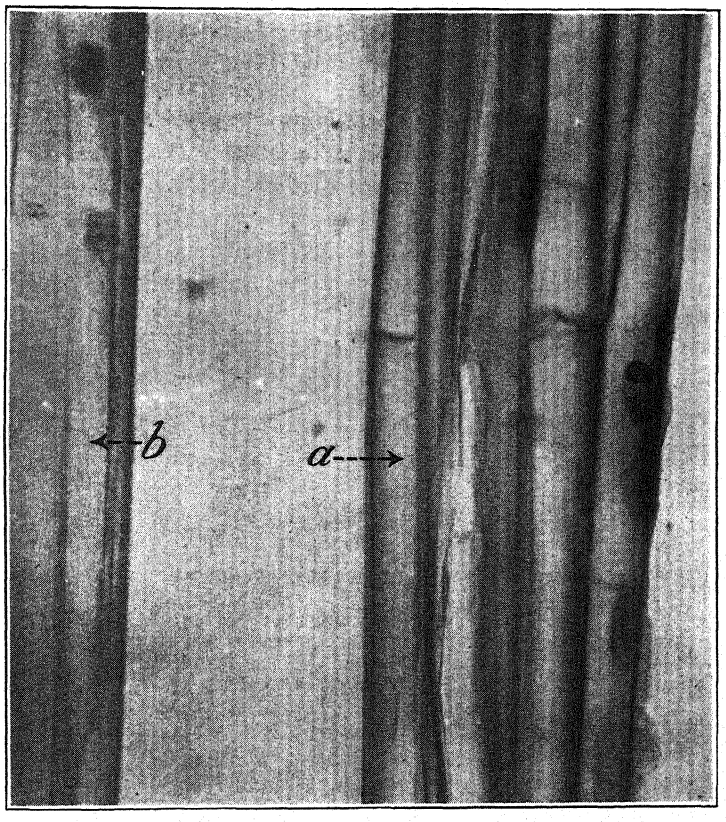


FIG. 2. A radial section from a Martin (nonsusceptible) plant about 7 days old, showing early stage of infection at *a* and wide interspace between coleoptile and *b* sheath of first true leaf (×525). Photograph by Dr. Erwin F. Smith.

cells the invading fungus seems to be gram-negative, as well as inert to both orange G and eosin. But at this stage the cell wall, the middle lamella, and the cytoplasm in the immediate vicinity of the point of entrance are decidedly gram-positive. Owing to this difference in standing reaction, the points of entrance were not recognized in the examinations made from 1919 to 1921, the hyphae being completely obscured by the dense violet color in the surrounding tissue. The notes on infection during the first two years' work, therefore, refer only to the second and third phases of development, in which the mycelium takes the violet stain and the surrounding tissue or cytoplasm reacts to the counter stain only. Therefore, it can not be assumed that when plants are recorded as noninfected no entrance of the fungus has taken place.

Hybrid 143, an extremely susceptible club wheat, Turkey Wn. 326, and Florence, moderately resistant, and two selections from Turkey Wn. 326 x Florence, produced by E. F. Gaines, of Washington State College, were studied for differences in resistance to bunt. The two selections from the Turkey and Florence cross showed results in many field tests indicating that they were much more resistant than either parent.

To eliminate to the utmost the errors due to small numbers, the two Turkey Wn. 326 x Florence selections have been combined in table 1 and treated as one variety, the two being very closely related and about equally resistant to bunt.

The sowings were made on November 10, 1919, and the plants emerged on November 20. Killings were made 6, 9, 16, 32, and 60 days after emer-

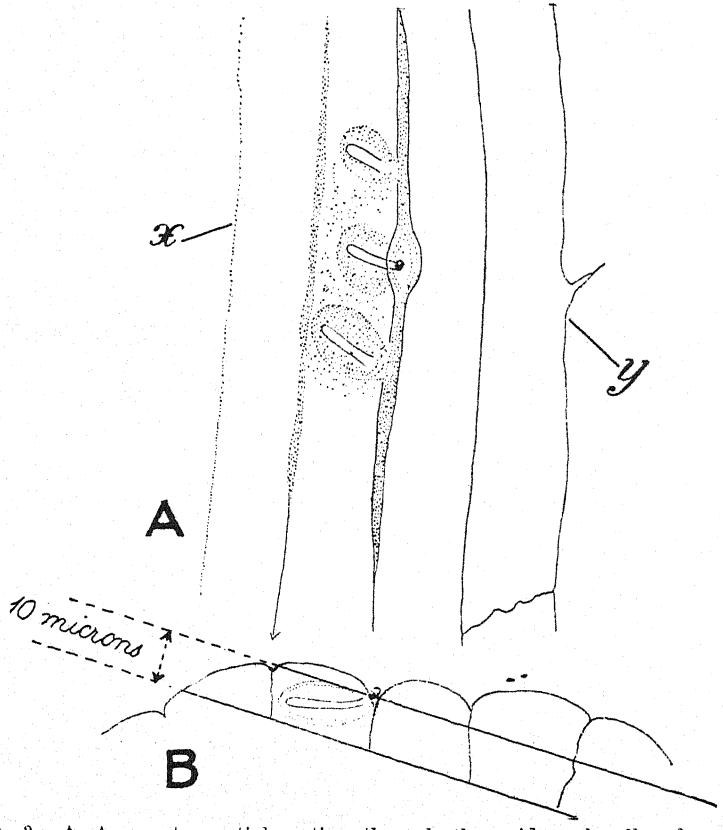


FIG. 3. A. A near-tangential section through the epidermal cells of a Hussar (highly resistant) plant about 7 days old, showing entrance of hyphae between walls of contiguous epidermal cells of the coleoptile and thence passing into the lumen. (Camera-lucida drawing, × 600.) B. A theoretical cross section of figure A on line *x-y*, showing point of entry of infecting hypha.

TABLE 1.—*Results of cytological studies showing percentages of plants found to be infected at definite periods of time after emergence, Corvallis, Oreg.*

Variety	Age of plant									
	6 days		9 days		16 days		32 days		60 days	
	+[a]	−[b]	+	−	+	−	+	−	+	−
Hybrid 143	15	10	8	8	5	5	8	0	16	9
Turkey Wn. 326	3	21	4	8	Lost		Lost		Lost	
Florence	0	25	4	20	Lost		12	0	0	10
Turkey Wn. 326 × Florence selections	8	17	4	16	15	9	7	16	2	9

[a] + indicates infection found.
[b] − indicates infection not found.

TABLE 2.—*Results obtained from plants grown to maturity in the greenhouse from the same sowings recorded in table 1*

Variety	Number of plants		
	Bunt-free	Partially bunted	Totally bunted
Hybrid 143	2	0	8
Turkey Wn. 326	9	0	1
Florence	8	0	0
Turkey Wn. 326 × Florence selections	55	0	0

TABLE 3.—*Results obtained from plants transplanted to field from sowings recorded in table 1*

Variety	Number of plants		
	Bunt-free	Partially bunted	Totally bunted
Hybrid 143	0	0	24
Turkey Wn. 326 × Florence selections	52	8	3

gence. Unfortunately, several of these lots were lost and others more or less damaged during the process of embedding, so that the record of examination is incomplete. Therefore, any attempt to draw definite conclusions from the percentage figure alone would be unsafe. Also, a wide margin should be allowed for experimental error in examination; that is, a failure to find a few bits of small faintly stained mycelium in the great mass of tissue searched.

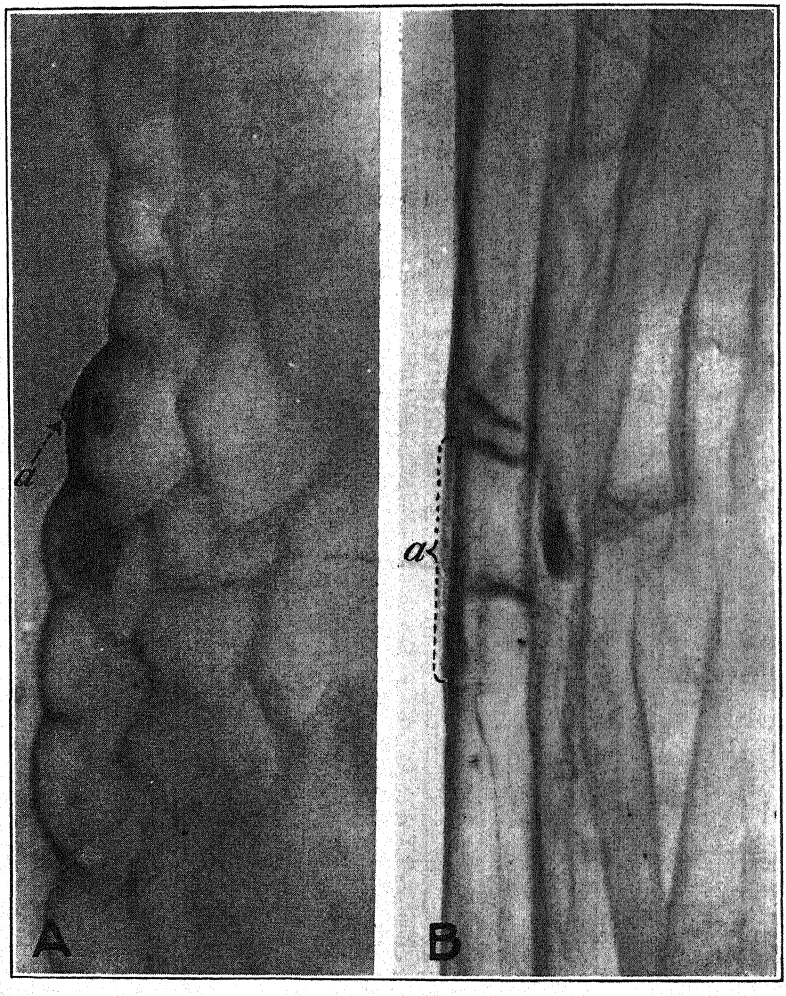


FIG. 4. A. A cross section of a Hussar (highly resistant) plant about 7 days old, showing characteristic thickening of epidermal wall at entrance and external hypha *a* (× 810). Photograph by Dr. Erwin F. Smith. B. A radial section from a Hussar plant about 7 days old, showing the sheaths enclosing the infecting hypha in the epidermal cell. An external hypha *a* is also shown. (× 810.) Photograph by Dr. Erwin F. Smith.

OBSERVATIONS ON NATURE OF INFECTION

The appearance of the infection in susceptible and nonsusceptible plants killed when 6 days old was in every way similar in both location and development. In all cases the fungus was confined to the coleoptile. The fungus also was confined to the coleoptile in seedlings of resistant varieties killed at 9 days of age, except in rare cases. In the case of Hybrid 143 it frequently had entered the first true leaf. When 16 days old the difference between the two classes is very apparent, both with reference to amount and location of the mycelium. In the Turkey Wn. 326 × Florence selections it had advanced in no case further than the contact between the sheaths of the first and second leaves and was not common; while in Hybrid 143 it was in many cases profuse and had penetrated into the second leaf sheath and into the subsurface internode. When 32 days old, the differences were still more pronounced, and, when 60 days old, the Turkey Wn. 326 × Florence selections, when infection was found at all, as a general rule, showed only small, slightly infected areas, which were seldom deeper than the third leaf. Plants from this sowing grown to maturity showed about the same percentage of infection as was found in the 60-day-old plants. In Hybrid 143, at the age of 60 days, the mycelium was found to be extensively and profusely distributed through the tissues of all the leaves and in the axis of the plant, and in some cases it had reached the growing point.

Apparently in the resistant variety, the suppressive action begins about the time the mycelium passes into the first true leaves or soon after the plant is 9 days old; and, except in rare instances, it ceases to be a menace to the plant by the time it is 60 days old.

Hybrid 143, Hussar (C. I. 4843), and Martin (C. I. 4463) were employed in the studies begun in 1921. The first variety is highly susceptible and the other two are apparently immune, as they had previously failed to produce even a trace of bunt in repeated field tests. Sowings were made in infested soil, as previously described, at a depth of 5.5 cm. The seedlings were killed for examination at the time of emergence and 7, 12, and 20[9] days thereafter. The method of staining was the same as that outlined in the foregoing pages except that the intensity of the gentian-violet-gram stain was reduced and the sections cut to a thickness of not more than 10 microns and generally as thin as 6 microns.

The examination of 60 plants of Martin, 40 of Hussar, and 20 of Hybrid 143 showed that the fungus entered the epidermis of the seedlings of both susceptible and resistant varieties apparently with equal facility. In all plants killed when 7 days old numerous points of entrance were found. As a general rule, no fewer than 100 points of attack per plant would be found.

[9] Ages only approximate, as plants vary at least 24 hours in emergence.

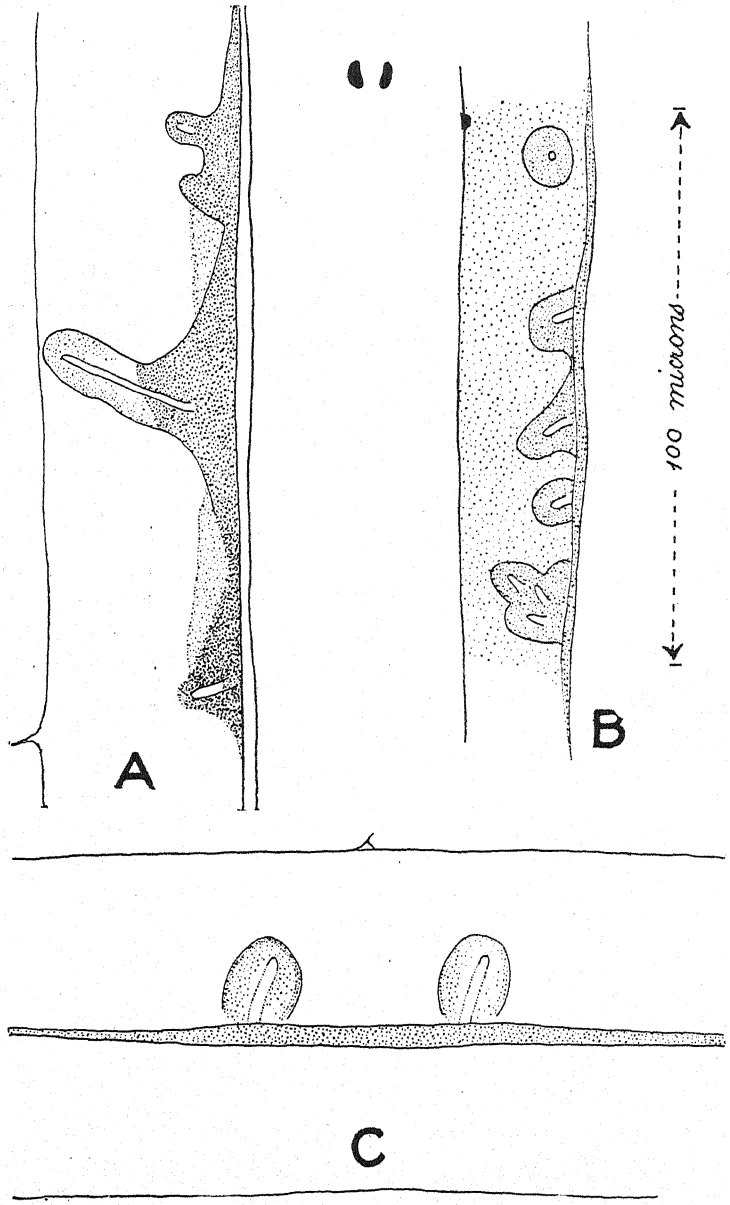


FIG. 5. Near-radial sections from Hussar plants about 7 days old, showing hyphae with enclosing sheaths in epidermal cells of coleoptile. (Camera-lucida drawings: A, ×1,000; B, ×730; C, ×800.)

In plants killed at the time of emergence but few points or groups of points of attack, usually 2 or 3 per plant, could be seen. But no plant was found to be entirely free. Evidently infection begins at about the time of emergence. This, however, may not be true for all environmental conditions of soil moisture, temperature, etc. In 25 per cent of the plants of Hybrid 143, killed when 7 days old, the second phase of infection was found. In Hussar and Martin, the second phase was found in only 5 per cent of all plants examined up to 20 days of age. In fact, so far as these examinations have progressed, it seems that in these two resistant varieties, the infection rarely develops beyond the first phase. No difference could be detected between plants 7 and 20 days old. It seems, therefore, that an inhibiting factor comes into action as soon as the hypha enters the lumen of an epidermal cell of the coleoptile. There is some reason to believe that even the most susceptible varieties possess, in some degree, such an inhibiting factor. This is suggested by the fact that in 70 plants of Hybrid 143 examined from 1919 to 1923 between the ages of 7 and 20 days never more than four distinct areas of second-phase infection have been found in the same plant and rarely more than two. These areas were in most cases sufficiently distinct to indicate that they had developed from separate entrances. The absence of the second phase of infection in other parts of the plant seems to indicate that at many points of entrance the fungus had failed to develop this phase. It, therefore, seems that some inhibiting factor or factors have prevented the development of the second phase. What these inhibiting factors are is as yet an unsolved problem.

TABLE 4.—*Results of cytological studies of wheat plants grown in bunt-inoculated soil and killed at definite ages, 1921 to 1923, Corvallis, Oreg.*

Variety	Age of plants when killed			
	At emergence	7 days	12 days	20 days
Wn. Hybrid 143	Five plants examined; 1st phase infection found in all; 2nd and 3rd found in none	Eight plants examined; 1st phase found in all; 2nd phase in 2; 3rd phase in none	Seven plants examined; all phases found in all plants	None examined
Martin, C. I. 4463	Fifteen plants examined; 1st phase found in all; 2nd and 3rd phases found in none	Fifteen plants examined; 1st phase found in all; 2nd and 3rd phases found in none	Fifteen plants examined; 1st phase found in all; 2nd phase in 2; 3rd phase in none	Fifteen plants examined; 1st phase found in all; 2nd phase in 1; 3rd phase in none
Hussar, C. I. 4843	Ten plants examined; 1st phase found in all; 2nd and 3rd phases found in none	Ten plants examined; 1st phase found in all; 2nd and 3rd phases found in none	Ten plants examined; 1st phase found in all; 2nd phase found in 1; third phase found in none	Ten plants examined; 1st phase found in all; 2nd phase found in 1; 3rd phase found in none

Location of Entrance.[10] About 75 per cent of the points of attack are found within 2.5 cm. above the seed from which the plant grew, notwithstanding the fact that the soil was evenly infested from the seed to the surface.

Entrance Phenomena. As has been stated by Dastur, the infecting hypha generally enters between the vertical walls of the epidermal



FIG. 6. A tangential section through coleoptile of a Hybrid 143 (susceptible) plant 15 days old, showing profuse second-phase infection (×520).
Photograph by W. C. Mathews.

[10] Previous experiments have shown that wheat plants can be grown through 1½ inches of sterile soil into an infested soil and yet produce bunted heads. That is, effective infection can occur more than 1½ inches above the seed. It also was found that plants can be grown in sterilized soil until the second true leaf appears and then become infected by transferring to infested soil.

cells, but occasionally it passes directly through the epidermis into the lumen of the cell. This can be demonstrated in cross sections (Fig. 4, A).

It is the opinion of the author that, after penetrating between the cells, the hypha usually enters the lumen of one of the adjacent cells (Fig. 3). This point, however, is sometimes hard to demonstrate in longitudinal sections, and sufficient examinations in cross sections have not been made to justify definite conclusions.

The first indication of an attack along the cell wall, when viewed in a longitudinal radial section, is a thickening of the epidermis for a distance of 20 or more microns which is highly gram-positive. When seen in tangential section, it appears as a circular spot which sometimes exhibits zonation. This thickening is apparently due to gelatinization of the portion of the epidermis lying between the cuticle and the plasma membrane (Figs. 1, 2, 3, and 4, A). A little later a conical protuberance is seen on the inside of the epidermis just beneath the point of attack by the hypha. In some cases this conical portion appears to be an indentation of the epidermal wall, as if the wall had yielded to mechanical pressure. However, this probably is not the case, as in later stages the hypha is seen to pass through the wall without any displacing effect. On the other hand, the entry of the hypha between cell walls, as seen in a tangential section, does produce a lateral displacement (Fig. 3). With the growth of the hypha this conical protuberance becomes, first, more or less globular (Figs. 2 and 4, A) and, finally, an elongated cylindrical sheath of from 8 to 12 microns in diameter enclosing the hypha, which has a diameter of 1 micron or less (Fig. 4, B). This sheath is probably what Wolf[11] observed in his studies of infection by *Urocystis occulta* (Wal.) Rab. and called a cellulose sheath. No photomicrograph has yet been obtained which shows the actual hypha within the sheath. It can, however, be seen plainly under the microscope (Figs. 3 and 5).

No adequate investigation of the physical or chemical nature of this sheath has been made, but, because of its intensely gram-positive reaction, it apparently is not cellulose. Usually, though not always, the space enclosed by it seems to take a deeper violet than the surrounding space, but this appearance probably is deceptive and the apparent color due to the fact that the interior is seen either through the sheath or against it as a background in the very thin microtome sections. Invariably there is a gram-positive condition at the point of entrance and its immediate vicinity.

There is apparently a decided tendency for points of penetration to appear in groups. Sometimes they are scattered irregularly over an area of 1 to 3 sq. mm., as seen in figure 1, A, but more often they are found at close

[11] Wolf, R. Der Brand des Getreides sein Ursachen und Verhütung. Halle, 1874.

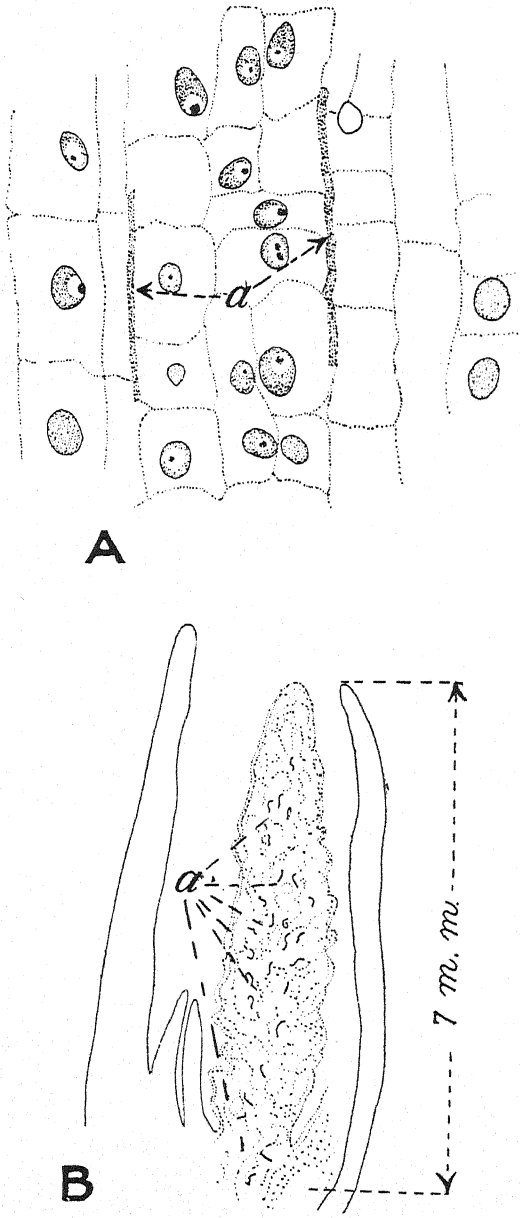


FIG. 7. Camera-lucida drawings showing third-phase mycelium in a Hybrid 143 (susceptible) plant 90 days old. A. Intercellular hyphae at *a* in the blade of one of the youngest leaves. Note normal appearance of the host nuclei. (×1,000.) B. Section through the embryonic head of the same plant (×100). *a* Indicates fragments of mycelium of which 25 were counted in this section.

intervals along the vertical cell walls, where the entering hyphae apparently spring from an external hypha lying along a cell-wall depression of the coleoptile (Fig. 5). An external hypha is seen in figure 4, B, and in cross sections in figure 4, A, but in this case it is between depressions.

The transition from the first to the second phase of infection is as yet obscure. The second phase in which the hypha becomes gram-positive is very rarely found in the epidermal cells, but frequently is found in the second cell layer near the points of entrance. The actual change has not yet been observed.

It seems a reasonable assumption that the change from first to second phase, that is to say, from gram-negative to gram-positive, is coincident with its establishing parasitic relations with the host.

Many questions regarding the process of infection and related phenomena remain for investigation, such, for example, as the chemical changes within the host and the influence of such environmental conditions as temperature and moisture in proximity to the seedling, pH concentration of the soil, and the presence or absence of toxic inhibitive substances or organisms resident within the soil. On these questions various hypotheses suggest themselves, but the object of this paper is to state observed facts rather than to indulge in speculation. The investigation should be continued. Its continuation by the writer was prevented by his retirement on account of age. It is now published in the hope that it may be a help to some younger investigator. The writer will gladly answer, in so far as possible, any inquiries from any one pursuing similar research.

SUMMARY

The process of infection of wheat seedlings by *Tilletia tritici* can be divided into three phases.

First: The entrance of the hypha into and its development within the epidermal cell, in which phase it is gram-negative and both intercellular and intracellular.

Second: Its development in the deeper parts of the coleoptile and in the sheath tissues of the earliest true leaves. In this phase, it is gram-positive and both intercellular and intracellular.

Third: Its development in the very young leaf blades and in the nodes, internodes, and the growing points of the plant, where it is gram-positive and strictly intercellular.

The fungus enters seedlings of both resistant and susceptible varieties of wheat with equal facility, but, in the most resistant varieties studied, apparently it does not develop beyond the first phase above mentioned, except in rare instances.

In varieties a little less resistant, the fungus develops the second phase of infection about as readily as it does in the susceptible varieties until the seedling is about 9 days old, after which it is retarded, and, in a high percentage of cases, ceases to be a menace to its host by the time it is 60 days old. In all varieties thus far examined, the entrance of the hyphae produces a gram-positive condition in the cell walls and cytoplasm in the immediate vicinity of the points of penetration.

TWO HARBORS,
MINNESOTA.

BACTERIAL SPOT OF RADISH AND TURNIP[1]

HAROLD E. WHITE[2]

INTRODUCTION

A previously undescribed bacterial-spot disease of radish and turnip has been found in Indiana (10). It is caused by a yellow monoflagellate bacterium which, in inoculation tests, has proved pathogenic not only to radish, turnip, and a number of other cruciferous plants, including cabbage, kale, cauliflower, and Brussels sprouts, but also to tomato, tobacco, and pepper. Its host range and cultural similarity to *Bacterium campestre, Bact. campestre* var. *armoraceae,* and *Bact. vesicatorium* raised the question of its relationship to these organisms. The radish and turnip organism is tentatively designated herein as *Bact. vesicatorium* var. *raphani.* A description of the symptoms produced on the various hosts, a greenhouse inoculation study of its pathogenicity, a comparative cultural study of the causal organism, and proof of its transmission with radish seed are reported in this paper.

The bacterial-spot disease of turnips was found by M. W. Gardner at Matthews, Indiana, in June, 1928. In July the writer found a similar disease on radishes growing in a greenhouse at Purdue University. The radishes were so heavily infected that the leaves were being rapidly killed by petiole lesions. In August, 1925, Gardner (2) found a disease near Indianapolis, Indiana, causing small black lesions on radish leaves, stems, and seed pods. Although an Alternaria was associated with these lesions, the symptoms of this disease agreed closely with those of the bacterial spot of radish, and very likely it was the same disease. A search of the literature has not revealed any previous description of a bacterial-spot disease of radish or turnip caused by a yellow organism.

CAUSAL ORGANISM ISOLATED FROM RADISH AND TURNIP

Isolations were made by macerating a portion of a lesion in sterile water on a flamed slide and plating out from this water in potato-dextrose agar by the loop-dilution method. Isolations were made from spots on leaves of turnip collected at Matthews, Indiana, and from radish at La Fayette,

[1] Contribution from the Botany Department, Purdue University Agricultural Experiment Station, La Fayette, Indiana. A thesis presented to the faculty of the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of Master of Science.

[2] The writer wishes to acknowledge his indebtedness to Prof. M. W. Gardner for suggestions and guidance in the development of this investigation and to Prof. E. J. Kohl and Mr. R. W. Samson for assistance.

Indiana. From these two sources and from all types of lesions the same type of yellow, rapidly growing bacterial colony was obtained. The pathogenicity of these two strains was proved by inoculation and reisolation and the purity of the cultures was proved by poured plates. No marked differences were noted between the two strains. Morphological, physiological, and cross-inoculation studies proved that the two strains were identical and either strain may be subsequently referred to as the radish organism. Greenhouse inoculations, to be described later, showed that mustard, cabbage, kale, Brussels sprouts, cauliflower, tobacco, pepper, and tomato plants were also infected by this organism.

SYMPTOMS ON RADISH, TURNIP, AND OTHER CRUCIFERS

In greenhouse-inoculation tests, the spots on radish leaves are irregularly circular, and are 2 to 3 mm. broad, sometimes larger. Very young

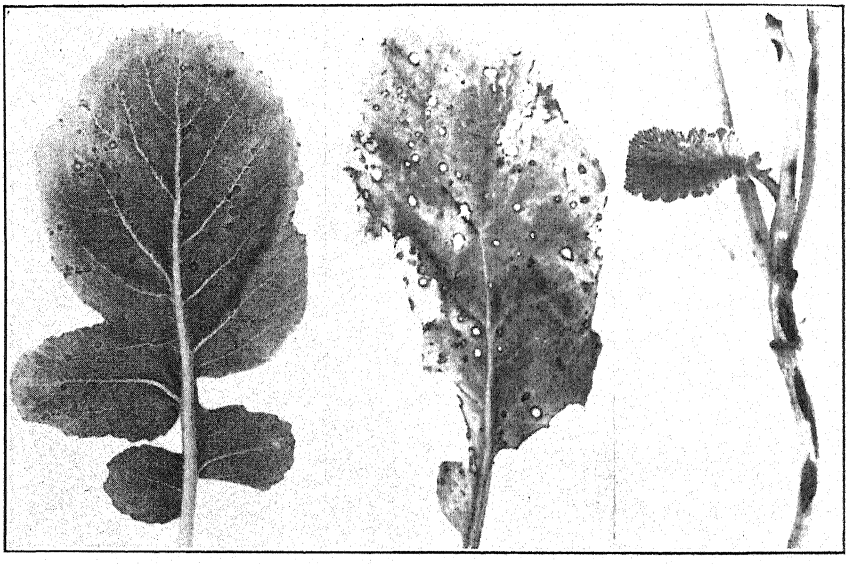


FIG. 1. Circular bacterial-spot lesions on radish leaves and elongated, sunken, stem and petiole lesions on radish seedling as a result of atomizer inoculation in the greenhouse.

spots appear as small circular black dots, later becoming light brown (Fig. 1). The larger lesions are light tan, sunken, slightly glazed, and surrounded by a narrow, yellowish, water-soaked zone, visible only upon close observation. The spots on the leaves frequently coalesce and become irregular in form but are not limited by the veins. Where the lesions are

numerous along the veins and midrib of the growing leaf, considerable distortion of the leaf blade occurs.

Petiole and stem lesions on radish are black, sunken, elongated, and 5 to 10 mm. long (Fig. 1). As the lesion increases in size it penetrates rather deeply and the petiole often breaks off at the point of infection. Petiole infection on young plants is the most destructive effect of this disease and with heavy infection an entire plant may be destroyed within 10 to 14 days after symptoms appear. Cotyledon lesions occur abundantly upon inoculated radish seedlings and plants from infested seeds. The cotyledon lesions are definitely circular in shape, 3 to 4 mm. in diameter, sunken, dark brown and water-soaked (Fig. 2, C).

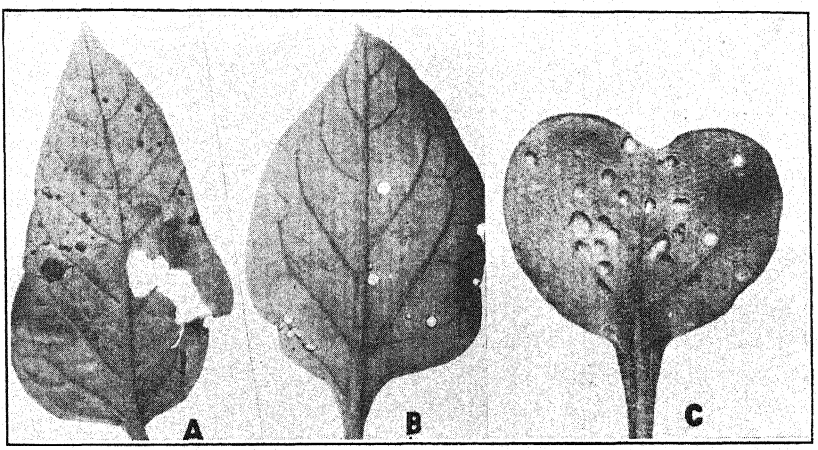


FIG. 2. A. Pepper leaf showing black lesions caused by *Bact. vesicatorium* from tomato. B. Pepper leaf showing circular whitish lesions caused by radish organism. C. Radish cotyledon showing circular lesions resulting from atomizer inoculation.

The lesions on turnip leaves and petioles are of the same general nature as those on radish. Cotyledon infection also occurs on turnip. The lesions on mustard leaves are at first small, black, circular spots, later 2 to 3 mm. in diameter, circular, greasy brown, and finally light tan. Petiole lesions are of the same nature as those on radish and are equally destructive on young plants.

The lesions on the leaves of cabbage, cauliflower, Brussels sprouts, and kale first appear as depressed circular black specks with water-soaked margins. As they increase in size the lesions remain circular and become more noticeably sunken, with a pale yellowish halo a millimeter or more in width. The lesions range from 2 to 4 mm. in diameter and, with age, change from a tan color in the center, surrounded by a purplish margin, to a chalky white. This chalky whiteness and the circular shape are the most striking

features of the lesions occurring on these hosts (Fig. 3). The petiole lesions on young plants of these hosts resemble those occurring on radish and turnip, and cotyledon infection is common. The petiole lesions do not occur so abundantly, however, and the disease is not so destructive on these hosts as on radish, turnip, or mustard plants.



FIG. 3. Lesions caused by atomizer inoculation with radish organism on leaves of cabbage and tomato seedlings.

SYMPTOMS ON TOBACCO, PEPPER, AND TOMATO

In greenhouse-inoculation tests the leaf lesions on tobacco plants are at first small, black, sunken spots, later chalky white with a slightly water-soaked border, and seldom more than 2 mm. in diameter when mature. Petiole infection was not observed.

Lesions on leaves of pepper plants are at first small, black, circular, sunken, water-soaked areas, becoming 3 to 4 mm. in diameter, dark brown, and later fading from brown to a chalky white (Fig. 2 B).

The lesions on tomato leaves first appear as circular black spots, later becoming 3 to 4 mm. in diameter, with light tan centers surrounded by a dark brown zone and, around this, a water-soaked margin (Fig. 3). Petiole and stem lesions on tomato are elliptical or irregular in shape, sunken, at first light brown, later black, and 4 to 8 mm. long. In the seedling stage the lesions often cut off the leaf or stem at the point of infection. Fruit lesions obtained by needle-prick inoculation are circular dark brown or black sunken areas about 5 to 10 mm. in diameter. The fruit tissue is invaded and blackened to a considerable depth under the lesion.

PATHOGENICITY OF CAUSAL ORGANISM

By means of inoculation tests, information was obtained concerning incubation period, mode of entry into host tissues, relationship to *Bact. campestre* and *Bact. vesicatorium,* host range, and varietal susceptibility. Cultures from two sources were used in inoculation work, one obtained from turnip at Matthews, Ind., and the other from radish at La Fayette, Ind. Results were confirmed by reisolation of the organism.

Incubation period and mode of entry. Leaf and petiole infection was readily obtained in the greenhouse by spraying very young radish and turnip plants with an atomizer containing a water suspension of the bacteria. The plants, after inoculation, were held in a moist chamber for 24 to 36 hours. In summer, lesions were visible within 4 to 5 days, but in winter, under greenhouse conditions, the inoculation period was as much as 10 to 15 days. The abundance of lesions obtained by atomizer inoculation indicated that wounds were not necessary for infection and that the mode of entry into the host tissues was through the stomata. Marginal leaf lesions occurred, probably as a result of hydathode infection, but were localized, and isolations made from tissue taken about one millimeter distant, along the veins, from the inner edge of such marginal lesions yielded no colonies and showed that vascular invasion had not occurred. The control plants, sprayed with water, remained free from infection.

Host range. In August, cabbage, cauliflower, kale, Brussels sprouts, and mustard were inoculated with the two strains of the radish organism by spraying with an atomizer. Five days from the date of inoculation lesions appeared on all the inoculated plants. Lesions occurred along the leaf margins, but there was no vascular invasion such as is characteristic of *Bact. campestre.* The noninoculated control plants of each host remained free from infection. Infection was obtained also on tomato, pepper, and tobacco plants in the greenhouse. Unsuccessful attempts were made to infect garden beans, soybeans, horse-radish, nasturtium, garden cress, shepherd's purse, and garden huckleberry (*Physalis* sp.).

Comparison with Bact. campestre and Bact. vesicatorium. Since the host range of the radish organism included hosts of two yellow organisms, *Bact. campestre,* the cause of black rot of crucifers, and *Bact. vesicatorium,* the cause of bacterial spot of tomato and pepper, it was necessary to compare the three organisms by inoculation as well as cultural tests. A culture of *Bact. campestre* was obtained from the University of Wisconsin and a culture of *Bact. vesicatorium* was isolated from tomato fruit. These two organisms and the radish organism were tested in a parallel series of inoculations on radish, turnip, mustard, cabbage, cauliflower, kale, Brussels sprouts, tomato, pepper, and tobacco. Infection took place on all the hosts

with the two strains of the radish organism. Vascular infection on cabbage was obtained with *Bact. campestre,* but no infection occurred on any of the other host plants. *Bacterium vesicatorium* infected pepper and tomato but none of the other plants. Under the conditions of these tests, the radish organism was pathogenic to a wider range of hosts than either of the other organisms.

Parallel inoculations were made on green tomato fruits with *Bact. vesicatorium* and the radish organism. Green tomatoes, gathered in the greenhouse, were washed with mercuric chloride solution, 1 to 1000, then with sterile water, and placed in sterile moist chambers. Punctures were made in the tomato fruits with a flamed needle dipped in a water suspension of the bacterial culture. The moist chambers with the inoculated fruits were kept at a temperature of 21° C. Seven to ten days after inoculation the fruits inoculated with the radish organism showed circular, sunken, black lesions around the punctures, 4 to 5 mm. in diameter, with an outer water-soaked zone often surrounded by a yellow halo. The inoculations with *Bact. vesicatorium* caused lesions closely resembling those formed by the radish organism.

Varietal susceptibility. A total of 42 seedsman varieties of radishes have been tested and all proved susceptible to infection. Likewise 9 varieties of turnips, 7 varieties of kale, 5 varieties of Brussels sprouts, 6 varieties of mustard, 6 varieties of cabbage, and 3 varieties of cauliflower were infected. No resistant varieties of any of these hosts were found.

MORPHOLOGY AND CULTURAL CHARACTERS OF THE CAUSAL ORGANISM

The organism is rod-shape and is motile by means of one polar flagellum. When stained with Van Ermengem's flagella stain, the cells appeared to have a thin capsule and varied in width from 0.65 to 1.2 μ and in length from 2.0 to 3.1 μ with an average of 0.96 by 2.49 μ. This is slightly in excess of the average length (1.94 μ) of the cells of *Bact. vesicatorium* (3). Otherwise the similarity is very close. Endospores and involution forms have not been observed.

The organism grows very readily on potato agar containing 2 per cent dextrose and no peptone and less vigorously on corn-meal agar or beef-peptone agar. The organism was grown in parallel series of cultures with *Bact. vesicatorium, Bact. campestre,* and *B. coli* on beef-peptone agar, potato-dextrose agar, gelatin, potato cylinders, milk, litmus milk, milk with brom cresol purple, milk with phenol red, nitrate bouillon, 2 per cent peptone solutions containing the different carbon sources, dextrose, saccharose, maltose, lactose, glycerin and mannit, nutrient agar containing these different carbon sources and the H-ion indicators, phenol red, brom thymol

blue, and brom cresol purple, nutrient agar with starch, Uschinsky's, Fermi's, and Cohn's solutions, blood serum, and sodium chloride solutions.

Gelatin was liquefied. No acid was produced in milk and no acid or gas was produced with the different carbon sources. Starch was dissolved. Nitrates were not reduced and no indol or skatol was produced from peptone. Growth occurred in Uschinsky's and Fermi's solutions but not in Cohn's solution. In all of these tests the radish organism closely resembled *Bact. campestre* and *Bact. vesicatorium*. It differed from the former in its more rapid liquefaction of gelatin, from the latter in its toleration of 4 per cent sodium chloride rather than 3 per cent, and from both in its failure to grow in Cohn's solution.

The radish organism grew well at 21°, 27°, and 32° C., and slowly at 12° C. and 35° C. The thermal death point in water suspension (6) was found to be between 50° and 52° C. Exposure for 20-, 30-, and 40-minute periods to bright sunlight greatly reduced the number of colonies in the unshaded portions of poured plates, and 60-minute exposures effected complete sterilization. The organism was not killed by a 45-day period of desiccation on glass.

TAXONOMY OF CAUSAL ORGANISM

The radish organism differs from *Bact. maculicola* McCulloch (7), the cause of a leaf-spot disease of cauliflower, and *Bact. angulatum* Fromme and Murray (1), the cause of angular leaf spot of tobacco, in that it is a yellow instead of white organism. *Bacterium tabacum* Wolf and Foster (11), the cause of tobacco wildfire, was found by Johnson, Slagg, and Murwin (5) to be pathogenic to pepper, tomato, cabbage, turnip, radish, and mustard, but also is a white rather than a yellow organism. *Bacterium melleum* Johnson causes a leaf spot of tobacco and was found pathogenic to tomato (4). However, the radish organism differs from *Bact. melleum* in being monoflagellate and in its failure to impart a honey-like color to potato-dextrose agar.

In culture the radish organism closely resembled *Bact. campestre* E.F.S. (9) but differs in host range and failure to produce vascular infection. The leaf-spotting *Bact. campestre* var. *armoraceae* McCulloch, recently described as the cause of a leaf spot of horse-radish, cabbage, and cauliflower (8), resembles the radish organism in type of lesion produced and in culture, even to a failure to grow in Cohn's solution but was reported not pathogenic to radish, mustard, or kale. Furthermore, attempts to infect horse-radish with the radish organism have been unsuccessful.

The radish organism closely resembled *Bact. vesicatorium* Doidge (3) in culture and in the type of lesion produced on tomato leaves. The lesions

formed on pepper leaves are, however, somewhat different, (Fig. 2, A and B). In parallel inoculation tests *Bact. vesicatorium* infected only tomato and pepper plants, whereas the radish organism infected radish, turnip, mustard, cauliflower, cabbage, kale, Brussels sprouts, and tobacco, in addition to tomato and pepper. In view of the fairly complete morphological and cultural similarity of these two organisms and their similar pathological effect upon tomato, it seems desirable for the present to consider the radish organism a strain of *Bact. vesicatorium,* which may be characterized as follows:

Bacterium vesicatorium raphani n. var.

Cylindrical rods, rounded at the ends, solitary, in pairs or chains; individual rods 2.0 to 3.1 μ by 0.65 to 1.2 μ; motile by a single polar flagellum, occasionally bipolar; aërobic, no spores; not conspicuously capsulate. Surface colonies on potato-dextrose agar, round, pulvinate, smooth, glistening, barium yellow, margin entire. Gelatin rapidly liquefied; no acid produced in milk; casein digested; nitrates not reduced; no acid or gas produced in media with various carbohydrates; gram-negative. Group number 211.3332513, or, according to the chart issued in 1920 by the Society of American Bacteriologists, 5322–31135–1333. Pathogenic on radish and turnip, forming lesions on leaf, petiole, cotyledon, and stem. Also pathogenic on cabbage, cauliflower, kale, Brussels sprouts, mustard, tobacco, pepper, and tomato plants and fruit.

TRANSMISSION WITH RADISH SEED

In the greenhouse work on varietal resistance, one lot of radish plants grown from seeds obtained from one firm showed infection before inoculations could be made, while seeds from two other firms, planted at the same time, produced plants with no signs of infection. Other lots of seed that had been on hand in the laboratory produced plants showing no signs of infection. Cotyledon lesions occurred abundantly on plants produced from the apparently infested lot of seeds and the causal organism was repeatedly isolated from these lesions. This evidence indicated that the organism was carried with radish seed and might be thus introduced into gardens.

More conclusive proof of seed carriage of the organism was obtained in tests with sterilized soil. In order to determine the possibility of control of the disease by seed disinfection, radish seed from seven varieties in the commercial lots found infested was immersed ten minutes in a 1–1000 solution of mercuric chloride and then planted in pots of sterilized soil. Nontreated seeds planted in similar pots served as controls. Counts were

made 28 days later to determine the percentage of infected plants in each pot.

Of the 40 plants from treated seed of the Early White Tipped variety, only 10 per cent were infected as compared with 38 per cent of the 42 plants from nontreated seed.

Of the 40 plants from treated seed of the Scarlet Globe variety, only 7 per cent were infected, while 44 per cent of the 45 plants from nontreated seed developed infection.

Of the 35 plants from treated seed of the Scarlet Turnip variety, only 8 per cent were infected. Fifty-eight per cent of the 43 plants from nontreated seed showed the disease.

The 40 plants from treated seed of the Early Giant Crimson variety showed 2 per cent of infection. Of the 40 plants grown from nontreated seed 17 per cent were infected.

Of the 36 plants from treated seed of the Woods Frame variety, only 14 per cent were infected, while 60 per cent of the 45 plants from nontreated seed developed infection.

The reduction of infection by seed treatment was still less in the two remaining varieties. Of the 38 plants from treated seed of the Early Round Red variety, 42 per cent were infected, while 62 per cent of the 40 plants from nontreated seed became diseased. Of the 50 plants from treated seed of Felton's White Box variety, 30 per cent were infected compared with 70 per cent of the 193 plants from nontreated seed.

The high incidence of infection occurring in the sterilized soil proved the disease to be seed-borne. Surface disinfection of the seed reduced the amount of infection but did not give a perfect control of the disease. Apparently, the organisms are so situated in the seed that not all can be killed by surface disinfection.

SUMMARY

The bacterial-spot disease of radish and turnip is characterized by black or brown circular lesions on the leaves and elongated, deep-sunken, black lesions on the petioles and stems of young plants.

The causal organism is a yellow monoflagellate bacterium, herein described as *Bacterium vesicatorium raphani* n. var.

While the disease is most serious on radish and turnip, particularly that following petiole infection, the organism is pathogenic also to cabbage, cauliflower, Brussels sprouts, kale, mustard, tomato, pepper, and tobacco. Tomato fruits also were infected.

On cabbage, kale, cauliflower, and Brussels sprouts the organism forms circular, chalky-white lesions on the leaves.

In inoculation tests a number of varieties of radish, turnip, Brussels sprouts, mustard, cauliflower, and cabbage were found susceptible and no resistant varieties were found.

The radish organism was compared with *Bact. campestre* and *Bact. vesicatorium* in cultural and inoculation tests. In culture the radish organism closely resembles *Bact. campestre, Bact. vesicatorium,* and, apparently, *Bact. campestre* var. *armoraceae.*

The radish organism differs from *Bact. campestre* in its wider host range and in type of symptom produced and from *Bact. campestre* var. *armoraceae* in its wider host range.

The radish organism differs from *Bact. vesicatorium* in its wider host range and type of lesion produced on pepper.

The organism was found to be carried on commercial radish seed with consequent seedling infection. Surface disinfection of seed afforded only a partial control.

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INOCULATION OF WHEAT WITH TILLETIA LEVIS (KÜHN)

E. W. Bodine and L. W. Durrell

Contrary to our usual conception of smut inoculation and infection, several investigators in the past have induced infection in cereals with different species of smut in ways differing from the normal.

As early as 1896 Hitchcock and Norton (4) used a pointed glass tube to inject a spore suspension of corn smut (*Ustilago zeae*) into different parts of corn plants and since that time this method of inoculating corn has frequently been used. Platz (7) succeeded in infecting corn plants by spraying them with a sporidial suspension and Griffiths (3) produced artificial infection of corn with conidial suspensions. The plants were inoculated either by injecting the conidial suspensions into the young parts with a hypodermic syringe or by pouring the suspension into the tops of the plants before they tasseled. Zehner (9), studying loose smut of barley (*U. nuda*), injected spore suspensions through the folded leaves and into or near the apical region of tiller growth, thereby producing smutted heads. Faris and Reed (2) produced loose kernel smut of sorghum (*Sphacelotheca cruenta*) in different parts of the plant by injection, though the only previously known means of infection by this organism was seedling penetration. Milan (5, 6), in studies with stinking smut (*Tilletia levis*), found that infection could be produced in plants past the susceptible period by puncturing the base of the culm with a needle and immersing the plant in a suspension of germinating chlamydospores.

The above results illustrate the prolonged susceptibility of the grain plants to smut infection. This also suggests that in the case of *T. levis* infection in the wheat plant might be produced after the seedling stage by introduction of inoculum.

In the following discussion the culturing of *T. levis* is described, together with an account of the use of both cultures and suspensions of spores in producing infection in plants beyond the seedling stage.

MATERIALS AND METHODS

A pure culture of *T. levis* secured from selected smut balls was first used. These smut balls were from local collections on the Experiment Station Farm, and, later, eleven other strains were cultured from smutted wheat from widely separated regions in the United States.

The smut balls were surface sterilized by dipping them into mercuric chloride 1:1000. They were removed and held with sterile tweezers, and with a sterile needle a small hole was carefully made in the smut ball and

spores quickly secured from the inside of the ball. These spores were then shaken onto nonnutrient agar in a petri dish. After five days the colonies resulting from germinating spores were transferred to nutrient agar and placed at room temperature for two to three days to bring out possible contamination. The cultures were then held at the more optimum temperatures of 12° to 15° C.

The fungus grows readily on several media and particularly well on Thaxter's potato hard agar. Growth is very slow at first, but in three to six weeks agar slants become well covered with a thin, snow-white, more or less prostrate, felty growth. When young, the colony is very white and smooth, with no indication of fluffiness or tufts of growth. As it gets older great masses of brown spores resembling chlamydospores are formed, giving the colony a brownish or tan color. This development or change of color occurs very rapidly in the light and at higher temperatures. This change also is accompanied by the development of the characteristic bunt odor. Microscopic examination shows the growth to consist of tangled masses of hyphae several hundred microns long, continuously producing masses of sporidia. The growth, however, is not so much by direct hyphal spread as by sporidial discharge. The sporidia are shot out from the main mass as described by Buller and Vanterpool (1) in the case of germinating chlamydospores. A veritable barrage of sporidia is discharged from the colony. These sporidia germinate on the agar where they light, producing small new colonies that repeat the process. Many sporidia are shot as far as the glass walls of the culture tube, where they stick.

A pure culture of *T. levis* is shown in figure 1, A, which illustrates the drifted manner of growth. It appears to differ materially from that figured and described by Sartoris (8).

Multisporidial cultures can easily be made from a culture with shooting sporidia by joining the flamed mouth of a tube containing a culture of *T. levis* with the flamed mouth of a flask of medium and wrapping the juncture in sterile cotton (Fig. 1, B). If left in this position two to four days sporidia are shot down to the medium below and there produce profuse colonies. This process can be repeated for weeks in making new cultures. These small white colonies can be used for inoculation or allowed to grow into a mat from which heavy suspensions of sporidia may be obtained for inoculation purposes.

Defiance wheat was used in infection tests. The seed was heavily treated with copper carbonate before planting in the greenhouse. This sterilization was effective, since no smutted plants appeared among several thousand check plants. Growth and maturity of the wheat plants were hastened by artificial light. The plants were grown in 5-foot rows and every other row was used as a check.

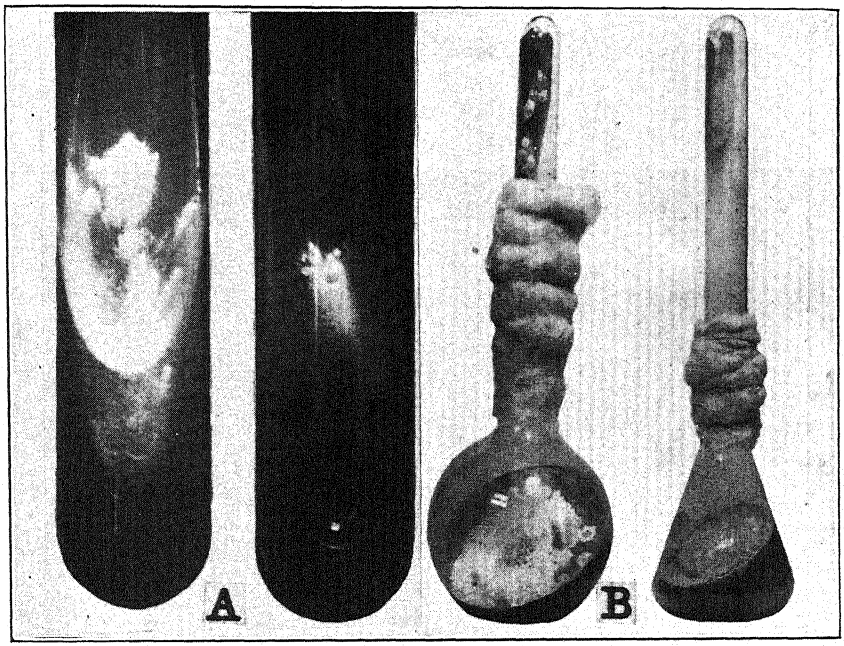


FIG. 1. A. Pure cultures of *Tilletia levis* on potato hard agar, showing the drifted character of growth by spore discharge. From material collected locally. B. Method of transfer by sporidial discharge from agar slants. Note the large colonies in flasks resulting from the great number of sporidia fallen from the culture above.

The wheat plants were inoculated at different heights, from 3½ inches to 18 inches. Three different kinds of inoculum were used, viz, small colonies six to ten days old resulting from germinating chlamydospores, spore suspension of germinating chlamydospores, and multisporidial cultures obtained by spore discharge consisting of hyphae and sporidia. The small colonies and multisporidial cultures were inserted inside the wheat sheath with an ordinary transfer needle. The spore suspensions were injected into the plant by the hypodermic needle. All inoculum was placed as near the nodes or the young growing wheat head as possible. Where suspensions were used chances of infection were probably greater because the one cubic centimeter of injected suspension filled the sheath and distributed the inoculum to the susceptible tissue.

INFECTION RESULTS

The above methods were used to inoculate 236 wheat plants in the greenhouse. For checks 322 other plants were used and, except for the inoculum, were treated as were the inoculated plants.

In the following table is a summary of the results obtained from inoculations of plants of different ages by inserting small cultures of *T. levis* into the sheath.

TABLE 1.—*Infection of wheat with Tilletia levis (Kühn)*

Height of wheat plants	Age when inoculated	Total number of plants inoculated	Percentage of smut
3½ inches	11 days	51	9.8
Control	11	75	0.0
6 inches	19	50	6.0
Control	19	50	0.0
8 inches	25	46	8.7
Control	25	65	0.0
12 inches	31	48	6.3
Control	31	71	0.0
18 inches	59	41	7.3
Control	59	61	0.0

Though the percentage of infection in the plants tested is not great, the results indicate that infection is possible by means of introducing colonies of *T. levis* in contact with the growing tissues.

The plants infected produced smutted heads many of which were distorted as in figure 2. Some of the inoculated plants showed similar distortion without visible signs of smut, though care was taken not to injure the growing stems when making inoculations.

Similar experiments also were made on plants 30 days old, 12 inches tall, grown from sterilized seed in sterilized soil. They were inoculated in three ways, viz, with small colonies, with a suspension of germinating chlamydospores, and with masses of sporidia and hyphae. The plants were held at 15° C. Only a few plants were inoculated but the results showed approximately 50 per cent infection. Adequate controls showed no infection. Inoculation of the flower and nearly mature head has failed so far. No boils so far have been produced on nodes or stems as reported for similar inoculation with *U. zeae*.

As might be expected, temperature plays a part in infection. In the first inoculation tests the temperature of the greenhouse was too high, being 20° to 25° C. Tests on a few plants held at 15° C. during the infection period showed a much higher percentage of infection. The temperature chambers were small and could only hold a few plants of wheat. A series including 15°, 20°, 25°, and 30° C. was tried with checks at 15° C. Three out of four of the plants inoculated with sporidial cultures produced

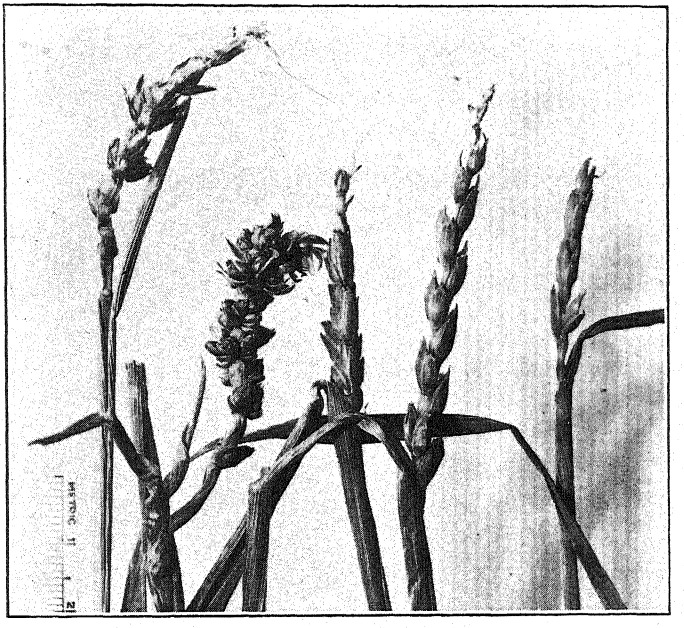


FIG. 2. Smutted heads of wheat produced by inoculation with cultures and spore suspensions. Dwarfing and distortion usually accompany smutting.

smutted heads at 15° C. and one out of three in those held at 20° C. Above these temperatures no infection was produced.

The reaction of inoculated wheat to temperature corresponds in a way to the behavior of the cultures at higher temperatures. Above 20° C. the organism in culture turns brown and produces spores which to all appearances are like the chlamydospores. At 25° and 30° C. the mycelium in culture breaks up and disintegrates.

The cultures *in vitro* follow about the same time schedule as does the fungus in the wheat plant, germinating, making vegetative growth, and darkening, with the production of brown spores in 50 to 70 days.

The wheat tissue appears susceptible to infection up to the time of flowering, though infection occurs more readily on the younger plants.

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FIELD OBSERVATIONS ON STRAWBERRY DWARF IN NORTH CAROLINA

NEIL E. STEVENS AND PAUL V. MOOK

The disease of strawberries discussed in the present paper has been well described by Plakidas[1] and by Brooks.[2] It is the most conspicuous malformation of strawberry plants in the Southern States, during the summer months, and has long been recognized by growers. In Florida the disease usually has been known as "crimps," but, in Louisiana and North Carolina, it is almost always called "dwarf." The name dwarf will be used in this paper both because it was used in connection with the first published description of the disease in this country and because it has the more general usage.

Brooks proved that the disease is due to a nematode. On the basis of examinations made of diseased plants submitted by Dr. A. N. Brooks from Florida, by Dr. A. G. Plakidas from Louisiana, and by Dr. G. M. Darrow and the writers from North Carolina and other Southern States, and after an investigation of the strawberry situation in and near Plant City, Fla., in March, 1929, Dr. G. Steiner of the Office of Nematology and Technology, Bureau of Plant Industry, concluded that the causal nema is *Aphelenchus fragariae* or a very closely related form. In view of the present importance of dwarf, it seems probable that the recent field observations in North Carolina may be of use to other investigators.

THE INTRODUCTION OF DWARF INTO NEW FIELDS

The desirability of new land for strawberry culture has long been recognized by growers. For this reason, strawberries are often planted in fields freshly cleared of timber. In such fields, even though well isolated from other cultivated fields, strawberries often show a high percentage of dwarf. One such field of the Klondike variety, near Chadbourn, N. C., showed well over 90 per cent of dwarf, while an adjacent field of strawberries of the same variety, separated only by a drainage ditch but planted with a different lot of plants, showed no evidence of the disease. The only reasonable conclusion is that the disease was carried to the fields in the strawberry plants themselves.

The following experiment was designed to test the importance of this means of distribution: A field at Chadbourn, N. C., was planted in February and March, 1929, with six lots of Klondike strawberries from widely separated sources. Examination for dwarf in October, 1929, showed the

[1] Plakidas, A. G. Strawberry dwarf. Phytopath. 18: 439–444. 1928.

[2] Brooks, A. N., J. R. Watson, and Harold Mowry. Strawberries in Florida. Fla. Agr. Exp. Sta. Bul. 204. 1929.

following percentages of the disease: Plot 1–0; Plot 2–0.5; Plot 3–0.3; Plot 4–0.8; Plot 5–5.0; Plot 6–5.0. It should be noted that the only lot of plants on which no dwarf was found came from a nursery north of the region where the disease has been found.

THE RATE OF SPREAD OF DWARF UNDER FIELD CONDITIONS

The results of a "census" of the dwarf plants in certain fields near Chadbourn on different dates during the summer of 1929 (given in Table 1) show the approximate abundance of the disease in that area. That a large proportion of the plants found diseased during the later inspection were already infected at the time of the earlier inspection is, of course, highly probable, and it is apparent that if roguing is to be attempted as a means of control, all but the least infected fields will have to be rogued more than once each season.

TABLE 1.—*Number of strawberry plants showing the dwarf disease in fields near Chadbourn, North Carolina, in 1929*

Field	Date of first inspection	Percentage of plants showing dwarf	Date of second inspection	Percentage of plants showing dwarf
1	June 24	13.8	July 29	16.6
2	June 24	15.7	July 29	13.7
3	July 2	14.0	July 30	15.9
4	June 24	10.0	July 30	15.0
5	July 6	17.4	Aug. 9	20.5
6	July 18	16.8	Sept. 9	23.0
7	Aug. 6	(about 15 per cent rogued out)	Sept. 9	7.5

INFECTION OF BLAKEMORE VARIETY AT WILLARD, N. C.

Attention has already been called[3] to the fact that this disease is spread by water. The circumstances surrounding the infection of the Blakemore variety at the Coastal Plain Test Farm at Willard, N. C., point so directly to the transmission of the disease by water that they will be given in some detail. Blakemore is the result of a cross between Missionary and Howard 17. It was developed by G. M. Darrow and G. F. Waldo of the Bureau of Plant Industry[4] at the U. S. Field Station at Glenn Dale, Maryland. Not only has this variety never been found infected with dwarf at Glenn Dale, but no evidence of the disease has ever been found there on any of hundreds of varieties in the extensive breeding plots. In addition to frequent inspections by the men in charge of the breeding work and by others

[3] *Op. cit.*

[4] Darrow, Geo. M., and Geo. F. Waldo. The Blakemore strawberry. U. S. Dept. of Agr. Circular 93. 1929.

interested in strawberries, the plots at Glenn Dale have been frequently surveyed by those most interested in this disease. Brooks spent several weeks there during the summer of 1926 and made a survey of the area in August, 1929. Plakidas visited these plots in the summer of 1928 and several times in 1929. It may then be safely assumed that the Blakemore plants were free from dwarf when sent to North Carolina.

The important information regarding the various fields of Blakemore at or near Willard is given in table 2.

TABLE 2.—*Date of planting, source of plants, and condition with reference to dwarf disease of the various fields of Blakemore at or near Willard, N. C.*

Field	When planted	Condition August 4, 1929	Condition October 16, 1929	Source of plants
A	Apr., 1927	No plants	No plants	Glenn Dale, Md.
B	June, 1928	Not examined	Apparently free	Field A
C	May, 1929	Free	" "	Field A
D	June, 1928	Dwarf present	Dwarf present	Field A
E	May, 1929	Dwarf present	Dwarf present (well scattered)	Field D
F	Mar., 1929	Free	Dwarf present in the lowest portion of the field	Glenn Dale, Md.

Field A—Well drained; land next to the grapes.
Field B—A field at Teachy (about 7 miles from Willard).
Field C—Well drained; land next to the grapes.
Field D—Low land next to railroad.
Field E, Field F—Part of a higher, but less perfectly drained, field than A or C.

It will be noted that the only fields found infected in August were D and the one (E) planted directly from it but that F also developed dwarf plants before October.

The location and history of these fields are significant. Field D, one of the lowest on the farm, is located next to a railroad embankment, bordering a small brook that drains all that section of the farm; E and F are really adjacent portions of a single field, higher and better drained than D; but F lies below E and there is a low place near the center of F through which the run-off from field E drained and where during the summer of 1929 water often stood. Dwarf plants are present only in the lower part of D, are scattered more or less irregularly over E, and are confined chiefly to the lower middle portion of F.

August and September, 1928, were unusually rainy in eastern North Carolina. Rainfall for September for the entire State was the heaviest since 1908; the combined rainfall for August and September, 1928, was greater than any recorded in the State. The August rainfall for Willard was 6.89 inches and for Wilmington 7.11 inches. The September rainfall for Willard was 9.02 inches and 13.41 inches for Wilmington. This cul-

minated in the tropical storm which swept over the eastern part of the State September 18 and 19, 1928, with a total of 4.40 inches for Willard and 4.76 inches for Wilmington.

Following these excessive rains, all the lower portion of field D was submerged for several days, as was a large part of the area just across the brook that was planted with seedling strawberries from Glenn Dale, reference to which will be made later. It is this submerged portion of field D which contains the plants now infected with dwarf. The only reasonable explanation is that the dwarf plants here were infected during the submerged period in 1928, that some infected plants were transplanted to field E, thus becoming scattered over the field, and that, subsequently, the lower portion of field F became infected from these plants.

That dwarf really is an infectious disease and not a condition due to poor drainage is indicated by the history of the seedlings mentioned above. These were so badly submerged by the storms of 1928 that most of them died and the survivors were subsequently planted on higher ground where certain patches showed in October, 1929, the most severe cases of dwarf that the writers have ever seen.

PROBABLE SOURCE OF THE INFECTION ON THE BLAKEMORE VARIETY AT WILLARD

Strawberries have been grown commercially at several places on the test farm at Willard, many of which were no doubt diseased. Throughout the seasons of 1928 and 1929, a field of about two acres of the Missionary variety, showing two to five per cent infection with dwarf, was grown on the test farm on an area that drains directly into the brook running beside field D. The planting stock for this field was obtained from an adjoining area, plowed up after fruiting in the spring of 1928. Fields A, C, E, and F do not drain toward D. The only apparent source of infection was the two-acre field of the Missionary variety from which water spread the disease to field D.

SUMMARY

Field observations in North Carolina indicate that the dwarf disease of strawberries is carried to new land by the planting of infected plants.

Typical fields of the Klondike variety near Chadbourn showed from 10 to 23 per cent of this disease during the summer of 1929. Several fields showed as high as 90 per cent.

Plants of the Blakemore variety originated at Glenn Dale, Md., which had never shown the disease, became infected during 1928 and 1929 at Willard, N. C.

The circumstances indicate that the disease was spread by water.

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EPHELIS-LIKE CONIDIA AND FLORET STERILITY IN ARISTIDA

WILLIAM W. DIEHL[1]

An abnormal specimen of *Aristida glauca,* gathered by B. C. Tharp in Lampasas County, Texas, in 1924, showed certain features in comparison with accompanying normal material which appear not to be recorded. This account deals with a short discussion of the abnormality and of the Ephelis-like fungus evident microscopically within the diseased spikelets of the grass.

THE DISEASED HOST PLANT

The diseased and normal plants are so unlike that it is difficult to recognize them as of the same species (Fig. 1, A). Grasses infected by species of Ephelis usually show dwarfing, but rarely so extreme as in this instance.

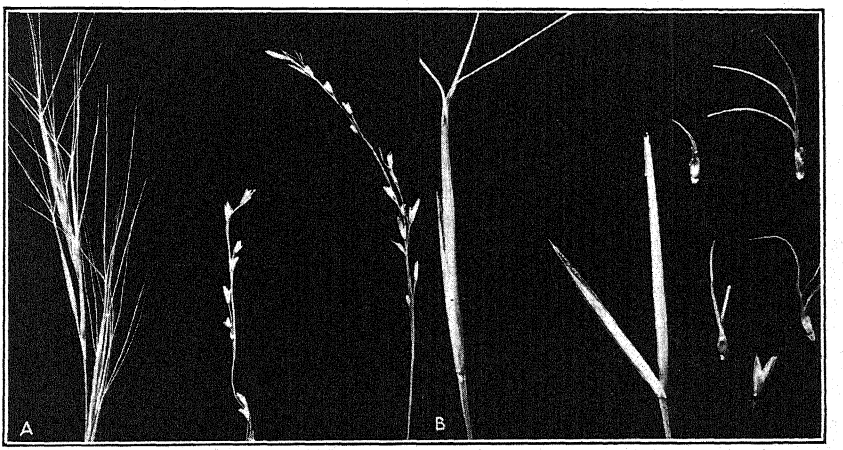


FIG. 1. A. (×1.2.) Normal inflorescence at left; diseased inflorescence at right. B. (×2.2.) Spikelets of Aristida: Left, normal spikelet; center, normal glumes, floret removed; right, diseased spikelets, at bottom, glumes only, floret removed.

In certain species of Ephelis the fungus surrounds the undeveloped inflorescence of the host which it encases, preventing thereby further floral development. In this instance, however, the diseased specimen is distinguishable superficially only by a consistent dwarfing of the entire inflorescence. Individual spikelets compared with normal ones (Fig. 1, B) are uniformly dwarfed. The glumes are found constantly to be approximately

[1] Thanks are due to Mrs. Agnes Chase and Dr. A. S. Hitchcock for verification of the grass host and to Mr. J. F. Brewer for the photographs.

one-sixth the length of normal glumes, although nearly as broad. The florets, which in Aristida are but one to the spikelet, are reduced in size proportionately. The grains failed to develop whether the coincident fungus mass was slight or extensive. A section (Fig. 2, A) through a

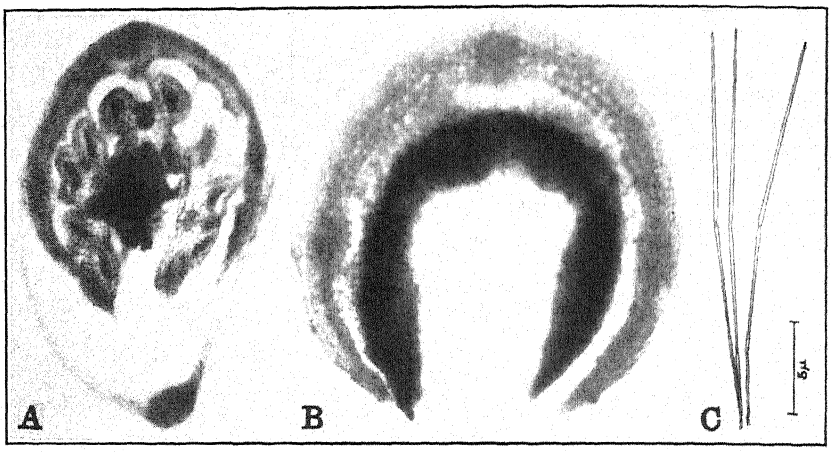


FIG. 2. A. (×78.) Free-hand section of diseased spikelet transverse to the main axis, showing inner glume and lemma enclosing stamens and undeveloped ovary with small central fungus mass. B. (×120.) Cross section—as in A—of a mature sclerotium, showing lemma partially enclosing the fungus layer with inner cavity lined by a mass of conidia. C. Interpretative drawing of conidia and conidiophores.

sterile spikelet with but slight fungous development shows little displacement of host tissues, although other sections (Fig. 2, B) where the fungous growth had developed extensively showed extreme displacement of host by fungous tissue to the extent of entire elimination of all the floret except the lemma.

THE FUNGUS

The fungus mass found in sterile florets may be regarded as sclerotioid, even though certain host tissues are included; this is homologous with the structure found in *Balansia hypoxylon* (*Ephelis borealis* E. & E.) and described by Atkinson[2] as a "pseudosclerotium." Mature sclerotioid masses where the floret tissues were largely displaced (Fig. 2, B), the lemma remaining as a rind, are quite variable in size, but none were observed over 2 mm. in length; they also vary in color from gray through brown to black. These mature bodies differ from sclerotia hitherto recognized in Ephelis in that here the entire central region is a cavity filled with a mass of acicular

[2] Atkinson, G. F., in The Genera Balansia, etc., p. 249. Jour. Mycol. 11: 248–267. 1905.

conidia of the type found in a normal Ephelis fructification. These conidia are of a dark greenish cast when in mass, but hyaline when single, 20–23 × 1–2 μ, rarely up to 29 μ long, borne upon short conidiophores not exceeding 3 × 1 μ. Although not clearly demonstrated, separation in suggested by the regular constriction and by frequent division into three segments. The protoplasmic content of these conidia is definitely guttulate (Fig. 2, C). The layer of spores presents the appearance of a palisade lining the lemma. They readily become detached from the conidiophores, fill the cavity, and extrude through the vertical aperture along the side not covered by the lemma.

The extreme variability in size and state of maturity of the fungus mass within the florets suggests a possible explanation for some obscure instances of floret sterility in other grasses. There should be no confusion, however, between this type of dwarfing and that dwarfing and malformation characteristic in Aristida when infected by species of Sorosporium. It may be significant in this connection to note that the writer's various attempts to culture living material of Ephelis on artificial media have been uniformly unsuccessful.[3]

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[3] After the above account had been sent to press the writer accompanied by Professor Tharp of Austin, Texas, visited the locality in Lampasas County on June 4, 1930, where these characteristically diseased plants are numerous. The writer is very grateful to Professor Tharp for transportation and other courtesies in this connection.



ASTEROCYSTIS RADICIS IN THE ROOTS OF CEREALS IN SASKATCHEWAN

T. C. VANTERPOOL

While conducting a systematic microscopic examination of the root systems of wheat, oat, barley, and rye seedlings grown in pots of soil collected from widely distributed localities of Saskatchewan, the writer was struck by the frequent occurrence of sporangia and hypnospores of *Asterocystis radicis* de Wild. (*Olpidium radicicolum* de Wild. (1) in the finer roots of oats and their occasional presence in wheat, barley, and rye. This fungus was observed also in roots of maize, western rye grass (*Agropyron tenerum* Vasey), and field mustard (*Sinapis arvensis* L.) seedlings in potted soil and in barley seedlings from the field. The pots were kept well watered throughout the experiments. As a rule, infection was slight and the roots appeared none the worse because of the presence of the fungus. Occasionally, however, infection of the oat roots was so intense (Fig. 1, C and D) that the affected roots showed signs of necrosis. In such instances the normal activity of the plants was doubtless greatly impaired, as evidenced by the slight yellow discoloration of the leaves of the seedlings. The most heavily infested soils appeared to be those with highest water-holding capacity.

In the light of the contributions of European investigators and more especially of the recent work of Guyot (4), these observations take on a new interest and perhaps warrant this note. According to this author de Wildeman reported as early as 1893 the presence of *A. radicis* in the roots of Gramineae. And Marchal observed this fungus on oats, in 1901, but never found it on wheat in the course of his experiments. More recently, other Continental workers (3, 5, 8) have observed its occurrence in oats and wheat. Rives (8), in 1925, attributes to this fungus the cause of an occasional root-scorch disease of oats in France and believes that the ability of the fungus to attack depends upon conditions unfavorable to the plant. Fron and Gaillat (3) were able to infect oats and several grasses with it but did not get infection on wheat. Guyot (4) could readily obtain infection of oat roots; but only in very heavily infested soil could he infect wheat. In the spring of 1926, he found *A. radicis* and other fungi associated with the roots of chlorotic and yellowed winter-wheat and oat plants obtained from low, wet spots in fields near Paris. Unlike Rives, he is inclined to believe that the scorch and chlorosis of these plants are not caused by *A. radicis* but are due to physiological disturbances brought about by excessive moisture. Peyronel (5, 6, 7) has frequently encountered

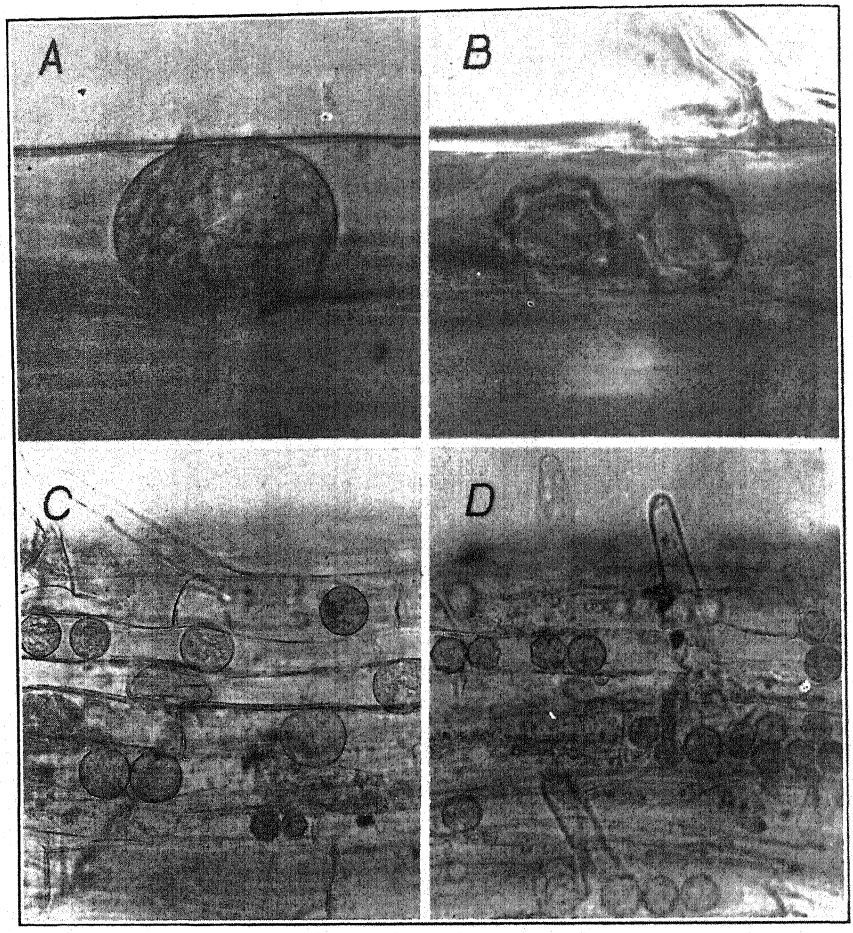


FIG. 1. *Asterocystis radicis.* A. Sporangium in an epidermal cell of an oat root with two very short exit tubes protruding slightly through the outer cell wall of the host. Stained with lacto-phenol-cotton blue. ×900. B. Two hypnospores in an epidermal cell of a wheat root. Photographed *in vivo.* ×900. C. Surface view of sporangia in an oat root. An empty sporangial sac is to be seen in the center of the photograph. Stained with lacto-phenol-acid fuchsin. ×280. D. Surface view of a portion of an oat root showing hypnospores in process of formation from sporangia (zygotes) and rounded-up zoöspores within the host tissue. Stained with lacto-phenol-acid fuchsin. ×280.

A. radicis on both healthy and decayed wheat roots while investigating mycorrhiza in that cereal, and he is of the belief that this fungus can become a virulent parasite under conditions unfavorable to the host. Guyot, in his recent contribution to the taxonomy and biology of *A. radicis,* contends that the characters hitherto used to separate *A. radicis* and *Olpidium bras-*

sicae (Wor.) Dangeard are no longer tenable and he strongly doubts whether the two forms can be maintained as distinct species. An exit tube for the discharge of the zoöspores from the sporangium was previously supposed to be lacking in *A. radicis,* but Guyot pointed out that the sporangia of *A. radicis* in oats and in *Poa annua* frequently possess one or more exit tubes. *A. radicis* has not been reported from America, but Guyot is of the opinion that the fungus described by Bensuade (2), in 1923, in the roots of tomato, tobacco, and cabbage in Wisconsin as *O. brassicae* is none other than *A. radicis.*

During his studies the present writer has frequently observed long exit tubes in the mature sporangia in oats, but less commonly in wheat. As is characteristic of *A. radicis,* the sporangia and hypnospores were found singly or in aggregates in the epidermal, root hair, and outermost cortical cells of the root (Fig. 1). The hypnospores varied in size from 8 μ to 40 μ in diameter, with an average of 18 μ (Fig. 1, B and D). The sporangia averaged slightly larger (Fig. 1, A and C). Occasionally, zoöspores which had been discharged internally would be seen in active motion confined in a host cell. The zoöspores were uniflagellate with the flagellum equal in length to about six times the diameter of the spore. Penetration of a root hair by a zoöspore was observed. (Fig. 2B) The zoöspore, however, is able to penetrate an epidermal cell of the root directly (*cf.* 9), as evidenced by the frequent presence of sporangia and hypnospores in epidermal cells just behind the root tip in the region where root hairs had not yet developed. Cells of the root cap also are sometimes invaded. The

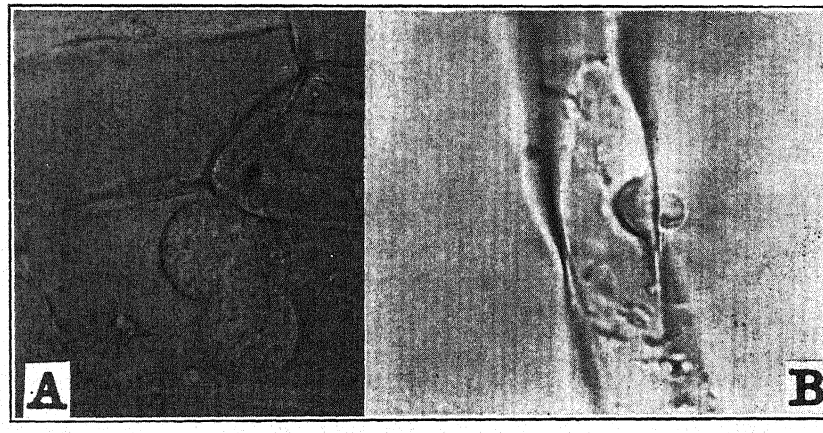


FIG. 2. A. *Asterocystis radicis.* At the top: An empty sporangial sac with papilla-like exit tube showing at the surface as a flattened ring. Below: Two immature individuals. ×900. B. *Asterocystis radicis.* A zoöspore penetrating a root hair of a wheat seedling grown in sterile water in a small flask. ×1350.

fungus shows up well in the host tissue when stained with lacto-phenol-cotton blue or lacto-phenol-acid fuchsin. Dilute Gram's iodine solution stains the sporangia bright brick red, while the hypnospores are stained a reddish brown. This reagent is also well suited for showing up the zoöspore flagellum.

It appears from the pot experiments conducted in this greenhouse that *A. radicis* is a normal inhabitant of Saskatchewan soils. It is doubtless maintained from year to year in the roots of various mustards and grasses as well as on the cultivated cereals. From the writer's findings and those of European workers it seems probable that only under exceptionally favorable conditions would this fungus cause any significant damage, and then only on oats. Alone, it need hardly be regarded as of any economic importance; but, as paving the way for or aggravating the damage caused by more vigorous parasites, it may doubtless become one of the many contributing factors in the root-rot complex of oats in some localities.

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PHYTOPATHOLOGICAL NOTES

A celluloid cell for inoculation of plants with insect vectors.—Cells of different sizes made of glass, wire mesh, and celluloid have been used by the writer in his work with tomato yellows, transmitted by a leaf hopper, *Eutettix tenellus* Baker. At first, various types of cells used by other workers were tried. Later, a modification of a celluloid cell was evolved that proved very convenient. The appearance of this cell when attached to the plant is illustrated in figure 1. It is about 5 inches in length and $2\frac{1}{2}$ inches in diameter. To make such cells, celluloid sheets 50 inches long and 20 inches wide were obtained and cut, each, into 24 equal pieces, approximately 5 x $8\frac{1}{3}$ inches in size. Glass bottles about $2\frac{1}{2}$ inches in diameter were used as forms to give these cells a cylindrical shape. While holding with one hand the small piece of celluloid so as to partly envelop the bottle, a cement was applied to the edges which were to lap. There are commercial cements on the market suitable for this purpose, but a good one may be quickly and cheaply prepared by dissolving pieces of celluloid in acetone. After smearing the cement along the edges of the two opposed surfaces the latter were pressed together and the cylinder, with its form inside, was laid on the table, seam side down, with some weight on the top. When the cemented parts harden in this position, a permanent union (Fig. 1, e), as well as a convenient shape, is obtained, but if taken off the form while the seam is still soft, the cylindrical shape of the tube may be lost. A piece of cloth of a desired density was fastened to one end of the cylinder by a couple of rubber bands and the space between these bands was saturated with the celluloid cement (Fig. 1, a). When this was dry, the superfluous edges of the cloth were trimmed close to the rubber band. A small hole was made in the center of the cloth for introducing insects. This was kept plugged with cotton when not in use (Fig. 1, b). At the other end, a cloth tube 4 or 5 inches in length and of the same diameter as the celluloid tube was affixed in a manner similar to the one just described (Fig. 1, c). The portion of the plant chosen for the exposure to insects is introduced into the cell through the open cloth end, which is then tied up around the stem of the plant with a string (Fig. 1, d). This cell was used in work with *E. tenellus* and *Paratrioza cockerellii* Sulc. and was found to be very convenient. No doubt, it may be advantageously used in connection with other insect vectors. It is very light and in most cases needs no support, except of the plant stem itself. In this respect it is much preferred to all kinds of glass cells and lamp chimneys. It permits the circulation of air, prevents excessive accumulation of condensation water, and does not obstruct the entrance of light. The enclosed plant parts and the insects may be

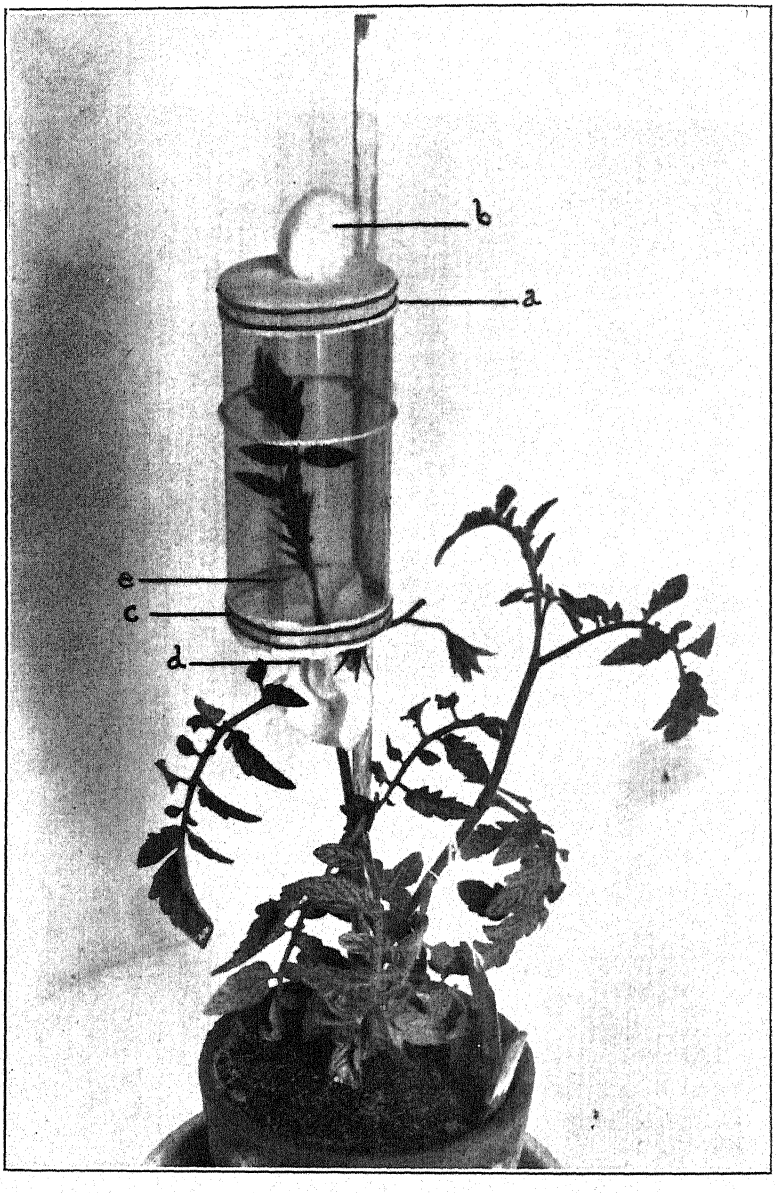


FIG. 1.—A small celluloid inoculation chamber attached to a growing tip of a tomato plant for exposure to viruliferous insects.

conveniently observed during the experiment. The insects may be readily located and removed with a suction tube. Finally, its size is such that, while giving plenty of room for the inclosure, it also allows an economic use of the material.—MICHAEL SHAPOVALOV, Western Tomato Disease Investigations, U. S. Department of Agriculture, Riverside, California.

Rhizoctonia on wild potato.—The small wild potato, *Solanum Jamesii,* occurs in the southwestern part of Colorado and adjacent regions. It produces small tubers that have been described as perfect miniature Early Ohios. Through the courtesy of Dr. Edna L. Johnson, of Boulder, the writer received a number of these tubers that bore black bodies having the appearance of sclerotia. Microscopic examination showed these black bodies to be sclerotia and later platings in all cases resulted in cultures of Rhizoctonia. The strain of Rhizoctonia obtained, however, has a growth habit differing from that of the stock laboratory cultures of *Rhizoctonia solani.*

The writer has been unable to find any reference to *Solanum Jamesii* as a host for Rhizoctonia. The occurrence of the fungus on the tubers of the little wild potato is interesting in the light of the prevalence of this organism in the soils of the State.—E. J. STARKEY, Colo. Agr. College, Ft. Collins, Colorado.

Note regarding a possible influence of soil reaction on development of powdery mildew on cowpeas.—Plant-pathological and soil-fertility workers quite often meet on common ground to study problems of mutual interest. Take, for example, powdery and common scab of potatoes, cotton wilt, and cotton rust; or, in the case of a deficiency in the soil of nitrogen, phosphorus, potassium, magnesium, manganese, iron, and other essential elements, pathological manifestations of one kind or another, either directly or indirectly, often are in evidence. The influence of soil, as to location and composition, is believed important by plant pathologists; also, the well-known effective control of certain diseases with materials like lime, or perhaps augmenting another plant trouble equally as effectively with the same treatment. The present note refers briefly to a case of relative susceptibility of cowpea plants to ordinary powdery mildew as influenced apparently by soil reaction.

During the season of 1929, an experiment was started at Arlington Experiment Farm to determine the comparative effectiveness of a number of phosphatic carriers, including superphosphate, lime or rock phosphate, soft phosphate, and dicalcium phosphate. The experiment is being conducted in an outside cage. Three plant indicators are employed, namely, wheat, cowpeas (Whippoorwill variety), and millet. These are grown in rotation in the order given. A duplicate series is included.

As it was desired to study the comparative effectiveness of the different phosphatic materials in a soil of varying reaction, a distinctly acid soil, pH 4.8, on Arlington Farm, was selected for the pot experiments. By the addition of hydrated lime, the reaction was changed to pH 6.4 and pH 7.5, respectively.

Without attempting to consider details pertaining to soil-fertility matters, attention is simply called to figures 1 and 2, which show the relative susceptibility of the cowpea plants to mildew under prevalent conditions. These specimens were taken from the nonfertilized soils. The matter is

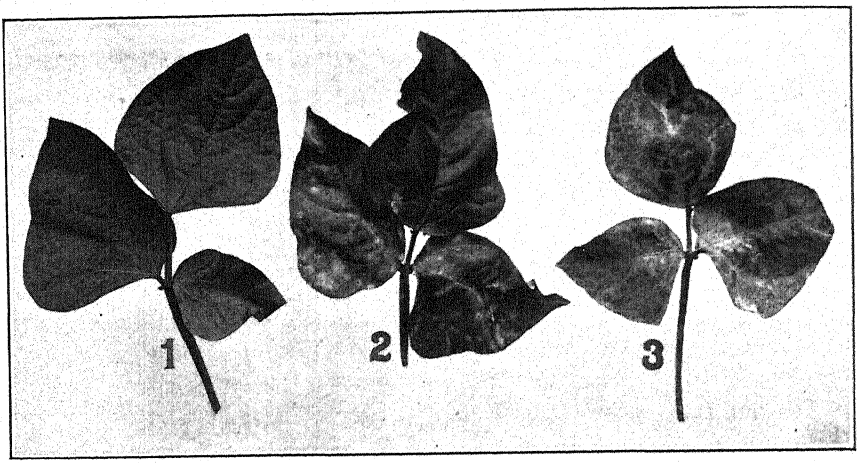


FIG. 1.—Showing possible influence of soil reaction on development of ordinary powdery mildew on cowpeas. No. 1—Soil reaction of pH 4.8. No. 2—Soil reaction of pH 6.4. No. 3—Soil reaction of pH 7.5.

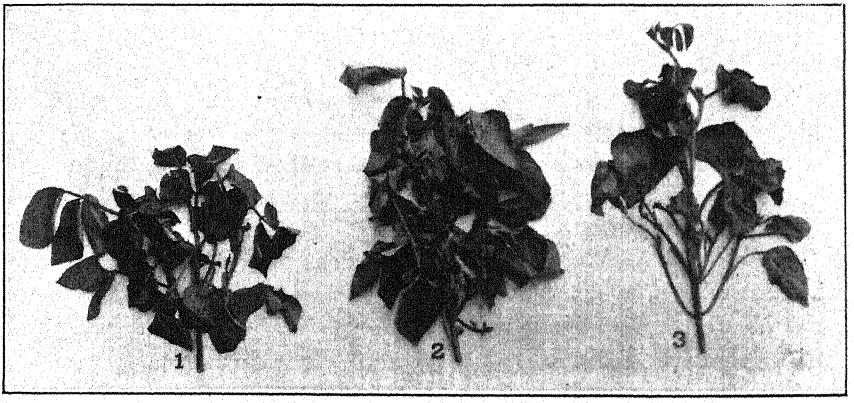


FIG. 2.—Larger specimens of cowpea material. No. 1—Soil reaction of pH 4.8. No. 2—Soil reaction of pH 6.4. No. 3—Soil reaction of pH 7.5.

recorded at this time because of the clear-cut manifestation of the disease under an alkaline-soil reaction and the equally clear-cut improvement shown with increasing soil acidity, and, furthermore, because it was felt the observation might be of interest to plant pathologists engaged in studying forage-crop diseases. In the case of cowpea plants grown on soil possessing a pH of 4.8 (No. 1), there was not evident a spot of mildew on a single leaf or stem. With a pH of 6.4 (No. 2), some mildew was found, and with a pH of 7.5 (No. 3), the ravages of the disease were quite marked, as indicated by leaves and stems.

Whether the soil acidity retarded the development of the disease through plant-sap changes, or whether the added lime may have influenced the character of the plant sap in such a way as to make it a more favorable culture medium for the organism, is not clear. No opportunity was afforded at the time to determine pH values of the respective plant saps or otherwise examine them for plant-food constituents.—B. E. Brown, Senior Biochemist, Soil Fertility Investigations, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

Notes on flooding injury to strawberries.—The strawberry section of southern Alabama is located around the towns of Castleberry and Brewton in the drainage basin of the Conecuh River. In March, 1929, this basin experienced a very severe flood which was far more extensive and caused much more damage than the previous great ones of 1861 and 1916.

The last of three periods of heavy rainfall during the month of February brought the rivers to flood stage and the saturated soil afforded an excellent foundation for the flood. The climax followed the enormous rainfall of March 13 to 15. At Brewton the precipitation during the later period was 16.26 inches, making a total of 24 inches in less than a month. The crest of the flood occurred on March 15. Parts of the region were inundated to a depth of 10 or more feet. The duration of the flood varied with the topography of the land. In general, the water had receded to such an extent by March 21 that no cultivated fields were submerged. The soil remained saturated, however, for an appreciable time because of additional rainfall from March 21 to 23.

The observations here reported are a part of the study of the strawberry-disease developments subsequent to the flood. The studies were made during the second week of April, approximately four weeks after the period of maximum precipitation. The Missionary variety is by far the most popular one in the Castleberry section, although both the Klondike and Aroma varieties are grown to some extent. At the time of the flood the majority of the plantings were in full bloom, the largest berries being slightly larger than peas. At the time of the writer's observation four weeks later

the plants, in general, were in full bearing. The period of time that elapsed between full bloom and full bearing was therefore prolonged approximately a week by the unusual weather conditions.

Most of he strawberry plantings were located on the uplands and, consequently, were not submerged. The heavy driving rains had caused deep gullies in the fields and had washed out many plants by the roots. A large part of the pine-straw mulch was washed from under the plants, leaving the berries in contact with the soil. They were thus directly exposed to infection by soil fungi. By far the greatest injury to the crop, because of the wet period, consisted in decay of the berries. In some fields as much as 50 per cent of the berries on the plants were partly decayed. A small percentage of the decay appeared to be hard rot, caused by *Rhizoctonia* sp. Most of it, however, exhibited the typical symptoms of leather rot caused by *Phytophthora cactorum* (Leb. and Cohn) Schroet.

In one of the lowland fields of Missionary strawberries from which the water had drained slowly, it was possible to observe the effect on the plants of various periods of submergence. The field was located on the sloping side of a lake having no visible outlet. At various intervals as the flood waters receded the water line was marked by the owner so that he was able to tell approximately how long each part of the field had been submerged. A few of the rows in the upper part of the field had not been flooded and, at the time of the writer's observation, were in excellent bearing. The adjacent rows on slightly lower ground appeared to be the same, although some of the plants had been covered for as long as two and a half days. No stakes marking the various water levels had been placed on the area which had been covered from six to fourteen days. Apparently no plants submerged less than a week were killed. The rows covered longer than a week contained numerous dead plants. The number of plants that were killed increased in proportion to the number of days of submergence, so that, near the stake marking the level on the fourteenth day, only a few of the mother plants having stout crowns were alive. There were no signs of life in any plant that had been covered longer than two weeks.

In a neighboring field Aroma strawberries had been planted between rows of high-bush blueberries, probably *Vaccinium virgatum* Ait. Water had remained in the depressions in the field and in some of these areas had killed both the strawberries and the blueberries. The zones of injured and dead plants indicated that the blueberries had about the same degree of flood tolerance as the strawberries.—Cyril O. Bratley, Bureau of Plant Industry, U. S. Department of Agriculture.

Field observations as an aid in avoiding diseased cotton seed for planting.—It is a generally accepted fact that cotton seed produced on dis-

eased plants, particularly plants infected with boll rots, are inferior to seed produced by healthy plants. If this be true, it should be possible to predict from field observations whether or not cotton seed produced in certain areas should be saved for planting. In the latter part of the season of 1928, the lower, or eastern half of the State of South Carolina, experienced an unusual amount of rain. Conditions were ideal for the development and dissemination of disease organisms. As was expected, these factors brought about an abundance of boll rots. Observations in the upper half of the State, where better weather prevailed, gave circumstantial evidence that the seed produced in the upper part of the State would be quite superior to that of the lower part. Germination tests of both upper- and lower-State seed were made to determine the planting value of seed from these localities.

Seeds were germinated in specially constructed germination pans. The same amount by weight of sand was placed in the pans and an equal weight of water added to each. Two hundred seeds of each sample were tested, the seeds being slightly pressed into the moist sand. Germination took place at a temperature range of 25° to 30° C. In these tests, all seeds with emerging root tip were considered as germinated.

Twenty-five samples of seed from the lower part of the State and 25 samples from the upper part were tested for germination. The average germination of the entire 25 samples of lower-State seed was 51.68 per cent, as compared to a germination of 79.64 per cent in the 25 samples of upper-State seed. The difference in the two lots of seed was 27.96 ± 1.1845.

Isolations from diseased bolls from several diseased fields gave an abundance of *Diplodia gossypina* and *Glomerella gossypii*. In most instances one or more species of Fusarium were obtained from the diseased material. None of these was *F. vasinfectum*, but seedlings grown in pots inoculated with the various cultures of Fusarium obtained from cotton bolls were considerably stunted in growth, due to root injury.

Diplodia gossypina was the most common pathogene developing on the seed in the germination pans. There was also a considerable amount of *Glomerella gossypii*, *Fusarium* sp., and various kinds of bacteria. None of seven morphologically distinct bacterial cultures, isolated from diseased bolls, gave evidence of parasitism on seedling cotton. However, many seeds which failed to begin growth in the germination pans were completely covered with a bacterial ooze. The symptoms of these decayed seed were typically those of a bacterial soft rot. Due to the fact that the writer left the experiment station in June, 1929, a detailed study of the organisms was not made.

Limited pathogenic studies indicated that several species of fungi and bacteria influence the germination and ultimate development of cotton seed

and seedlings, even though some of the organisms may appear saprophytic on the mature cotton boll.

The conditions existing in South Carolina in 1928 may have been unusual and may not recur soon. However, similar conditions can be expected in certain cotton-growing sections of the South—at least occasionally. The opportunity may arise in the future for other workers to make similar observations that will warrant certain recommendations to the cotton grower regarding the source of seed. For that reason the foregoing results are here presented.—JAMES M. WALLACE, Twin Falls, Idaho.

BOOK REVIEW

Hodgman, Charles D., and A. Lange Norbert. Handbook of Chemistry and Physics. 14th ed., 1386 pp. Chemical Rubber Publishing Co.: Cleveland, Ohio, 1929. Price $5.

In America the term applied mycology has never been approved as a proper appellation for the study of plant diseases, partly because in certain industrial connections it transcends the field of plant-disease control, but chiefly because plant pathology is so much more varied a science than mycology. Though the foundations of all phases of experimental botany are laid in chemistry and physics, phytopathology, in its more practical aspects, partakes also of the nature of an engineering science, and in this respect might be said to stand in about the same relation to plant physiology as industrial and engineering chemistry do to theoretical chemistry. This is due to the fact that so large a part of the service rendered by any applied natural science is for the purpose of establishing controls and correcting tendencies that have gone wrong. If an authoritative and reasonably complete reference book to chemical and physical data is indispensable equipment to experimental biologists in general, how much more essential is it for the plant pathologist?

The series of handbooks of chemistry and physics published by the Chemical Rubber Publishing Company has long been recognized by the unprofessional chemists, physicists and mathematicians, who constitute the majority of workers in experimental biology, as one of their principal supports in working with the resources and methods of these sciences. The latest edition has been expanded in certain directions that make it even more useful to the student who is concerned with the measurement of environmental factors and the responses of organisms. In fact, most of the chemical and physical data found in such encyclopedic works as the International Critical Tables and Smithsonian Tables that are of concern to the biologist and general laboratory worker are collected here.

Of special note among the subjects the treatment of which is new or completely revised are the following: A table of indicators, with an explanation of the properties thereof and showing the name, source, and pH range; the preparation of Clark and Lubs indicator solutions; conversion factors for E.M.F. to pH with the standard electrodes; electrolytic and oxidation potentials; properties of dry and saturated air including volume changes with temperature and pressure, heat capacity. etc.; similar data for air of various percentages of saturation; tables of heat of combustion of organic compounds, gases, and various substances, including coal, wood, and oil; tables showing the functions, uses, and compositions of foods; wave-length of prin-

cipal lines in the spectra of the elements; transmission of radiation by different neutral and colored glass; physical constants of resins and gums in addition to those of oils, fats, waxes, and inorganic and organic compounds generally; interest tables; a synopsis of each of the elements; examples of various volumetric reactions with gram equivalents.

The tabulated data of many other subjects too numerous to mention appear as in previous editions. Of special interest in the present relation are: Preparation and standardization of laboratory reagents, solubility of cane sugar, gases, and inorganic compounds in water, cuprous oxide equivalents of various sugars, hydrometer and thermometer conversion tables, vapor tension of water, psychrometric tables, photometric standards, photographic formulae, various weights and measures. An index of over 1000 entries affords easy accessibility to the diverse information given.—F. WEISS, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

PHYTOPATHOLOGY

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A NEW BACTERIAL LEAF DISEASE OF TOBACCO IN THE PHILIPPINES[1]

FELICIANO M. CLARA[2]

A bacterial leaf spot of tobacco, resembling wild fire in some respects, was observed in December, 1925, in the seed beds and afterwards in the field of the Ilagan Tobacco Experiment Station of Isabela. Later, the same disease was found in several localities of the Cagayan Valley. The spots on seedlings generally are opaque and without the characteristic yellow halo of the wild-fire disease. On full-grown plants the yellow zone may be found at times associated with some spots. Due to the similarity of the disease to wild fire, it was at first regarded as such. The result of subsequent studies, however, indicated a disease different from wild fire and similar but not identical to that reported by Johnson (5) as "Wisconsin bacterial leaf spot" caused by *Phytomonas mellea* (Johnson) Bergey *et al.* Since the disease affects the quality and quantity of the crop produced, any authentic information about its occurrence, nature, and possible suppression will be of some value to growers.

DISTRIBUTION, ECONOMIC IMPORTANCE, AND LOCAL NAMES

The writer is not aware of any report of the occurrence of this disease in other parts of the world. It probably has been present in the Philippines but has been confused with other leaf spots of tobacco because of the names applied to it. The different local names given these diseases can not be used to distinguish one from the other. In the Cagayan Valley and the

[1] Published in Phytopathology with the permission of the Director of the Philippine Bureau of Agriculture. The greater part of this work was done at the Coöperative Plant Pathology Laboratory of the Bureau of Agriculture and the Bureau of Science, Manila.

The writer acknowledges the coöperation of Mr. G. Merino, Dr. C. J. Humphrey, Dr. N. G. Teodoro, Mr. D. B. Paguirigan, and Mr. F. B. Serrano in obtaining materials while conducting this investigation in the Philippines.

[2] I am particularly grateful to Professor M. F. Barrus and Professor W. H. Burkholder, who kindly corrected the manuscript and gave invaluable suggestions, advice, and encouragement while completing this work at the Department of Plant Pathology at Cornell University. Dr. Burkholder has also given me the privilege of consulting his manuscript on the genus Phytomonas, including several important references.

Ilocano regions of the northeastern part of Luzon, the commonest local terms employed for leaf spots, irrespective of the symptoms and causes, are "palatao," "palatac," and "batec." These names have no more significance, even locally, than has the term "leaf spot."

In the Cagayan Valley, the disease was particularly serious in 1925, and similar troubles seem to have occurred previously. It is very evident, however, that nothing was known about it. Specimens of fresh tobacco leaves received from Jaro Iloilo, one of the Visayan Islands, showed that the disease was present also in that region. A systematic pathological survey probably would show a much wider distribution and give a better realization of the rôle played by the disease in the tobacco industry.

The damage caused by the disease, independent of the other kinds of leaf spots, such as frog eye, wild fire, angular leaf spot, and others, merits some attention. The extent of infection on seedlings in the seed beds of the Ilagan Tobacco Experiment Station during 1925 and 1926 and later transmitted to the field caused great concern. Seedlings grown in seed flats, although the soil employed was sterilized with formaldehyde, showed from 50 to 90 per cent damage. Presumably, the same trouble occurred in the seed beds of individual growers, as there was a great shortage of seedlings during that season. When infected plants were set in the field, a large percentage of the leaves at harvest showed the effect of the disease. Some were rendered nearly useless, while others fell into an inferior and cheaper grade. The damage varies according to the use made of the different classes of leaves. Spotted wrapper leaves can not be used for high-grade cigars because of their lack of uniformity in color and their poor texture. Such leaves can be used only as binders and fillers. But, even in such cases or however used, the disease lowers the quality of the product. The real effect upon the quality of the crop, in addition to other losses, does not receive proper recognition on account of the system of marketing in vogue, whereby no discrimination is made by the buyer. This practice gives no encouragement to produce a good quality of tobacco which can be brought about, other things being normal, through the control of disease. As to the actual reduction in the quantity of the crop, no estimate was made. There is, however, no doubt that the disease is a limiting factor, as shown by its seriousness both in the seed beds and in the fields.

SYMPTOMS OF THE DISEASE

In the seed beds. Young seedlings when severely infected develop a kind of wet-rot similar to bed rot (Fig. 1, A and B). The young plants may thus be wiped out and the condition easily confused with typical damping-off diseases. The presence of opaque or bleached white spots on

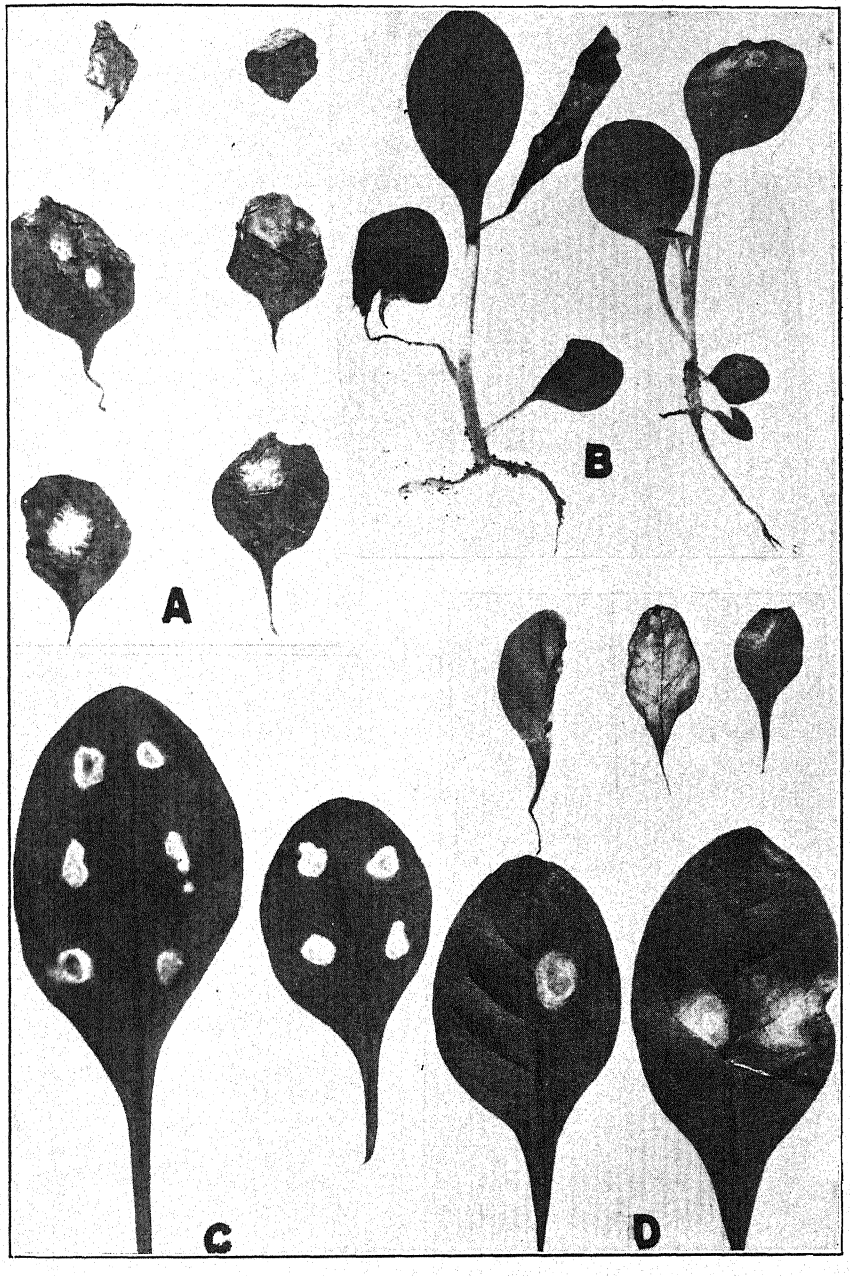


FIG. 1. A. Natural infections produced by **Phytomonas polycolor**, n. sp., on leaves of tobacco seedlings. B. Wet-rot form produced by inoculations through needle punctures. C. Inoculations with bacterial smear without punctures. D. Inoculations with bacterial smear through needle punctures. Natural size.

the leaves (Fig. 1, C and D) shows that the disease is actually different from ordinary damping-off or bed rot associated with such fungi as *Pythium debarynum* Hesse and *Sclerotium rolfsii* Rach. When infection starts on the edge of the leaves, a wet-rot becomes evident (Fig. 1, B). More apparent damage results when the infection occurs on the petioles of the leaves and on the stems, as it then rapidly kills the plant. The leaf spots vary from the size of a pin head to that of a "centavo," and there are cases where entire leaves are involved. The spots are visible on both sides of the leaves and sometimes holes are produced. Wild fire, which may also be found on seedlings in the seed beds as well as on older plants in the field, may be confused with this bacterial leaf spot, but a closer examination of the symptoms and characters of the causal organism readily reveals the distinction.

In the fields. Infection in the field is recognized by the presence of spots on the leaves, generally white or opaque, but sometimes brown and zonated, about which there is occasionally a narrow yellow halo. These spots may be of various sizes and may even coalesce to form larger irregular ones. The grayish center and the pustules generally present in frog eye are absent in these spots except when a secondary organism has developed there. Young lesions may occasionally possess a yellow halo quite similar to wild fire. In such cases it is very difficult to identify one from the other by external appearance. The yellow "halo" disappears as the leaves mature. The disease is more virulent on the lower than on the middle and upper leaves, possibly because of their greater susceptibility or to the more favorable conditions for infection proximate to the lower part of the plant. The brown spots are not easily seen on cured leaves because of the similarity in color to such leaves, but the white ones remain conspicuous.

CAUSE OF THE DISEASE

Isolation. The causal organism, among a number of other bacteria, was first isolated from diseased seedlings in the seed beds of the Ilagan Tobacco Experiment Station at Isabela during December, 1925. Hundreds of such isolations were made and the different kinds of bacteria obtained in culture were numbered and used for preliminary inoculations with the object of eliminating all that were not pathogenic to tobacco. Each culture was obtained from a single colony by employing the usual bacteriological dilution procedure in all the isolation work. One of these cultures, numbered 116-C31, which showed green fluorescence in broth, was found very pathogenic, while the others did not show any infection at all and were, therefore, discarded. This organism was later repeatedly isolated from freshly infected leaves of full-grown plants, from badly spotted leaves in the

curing sheds of the Experiment Station in 1925 and 1926, and from leaves received from Iloilo in 1927. It was obtained also from leaves that had been dried between papers and kept in the laboratory for more than a year. Some isolations made from seedlings that had developed a wet rot, typical of the disease, showed this same organism. These seedlings came from seeds that were germinated, without disinfection, on absorbent cotton in petri dishes and kept moist with sterile water. This seems to indicate the presence of the pathogen on the seeds.

The material from which isolations were made was first placed in 1-1000 mercuric chloride for 10 to 20 seconds and then rinsed several times in sterile water. It was then dropped in beef bouillon, +1 Fuller's scale, from which dilution plates were prepared the third day. Plating was made on potato-dextrose agar, +1. This gave an abundant growth after 24 hours under room condition and proved the best medium for obtaining a sufficient supply of inoculum.

Inoculations. Several inoculation experiments were made on seedlings and on plants at various ages up to the flowering stage. These plants were all grown on sterilized soil and kept in a building covered with wire mesh. Culture 116-C31, on potato-dextrose agar, +1, except in a few cases, was used for all of the inoculation experiments.

Various methods were employed, such as inoculating in punctures made with a sterile needle, spraying the host with a bacterial suspension in distilled water, and direct application of a bacterial smear.

When inoculations of seedlings were made in punctures and the pathogen applied in water-drop suspension, the infection was very rapid and frequently developed into a wet rot (Fig. 1, B). Even quicker results were obtained when bacterial smear was applied to the punctures with a 3 mm. platinum-wire loop. The infection was readily noticed after twelve hours, especially if the plants were kept under bell jars. Practically all parts of the plant—the stem, the petiole of the leaves, the youngest shoots—were easily infected, finally resulting in the death of the entire plant. Inoculation with bacterial smear, with and without punctures, readily showed infections (Fig. 1, C and D). The check plants, although punctured and placed under the same conditions, except the application of the inoculum, remained normal.

On nearly full-grown plants and at other stages, inoculations made by applying a smear of the culture to the leaves and locating them where desired to facilitate detection of the infection proved to be satisfactory for comparative purposes (Fig. 2).

In all cases, both inoculated and control plants were kept under bell jars from 12 to 24 hours. The infection was noticeable on the inoculated points on the second day, and the spots were invariably opaque or white.

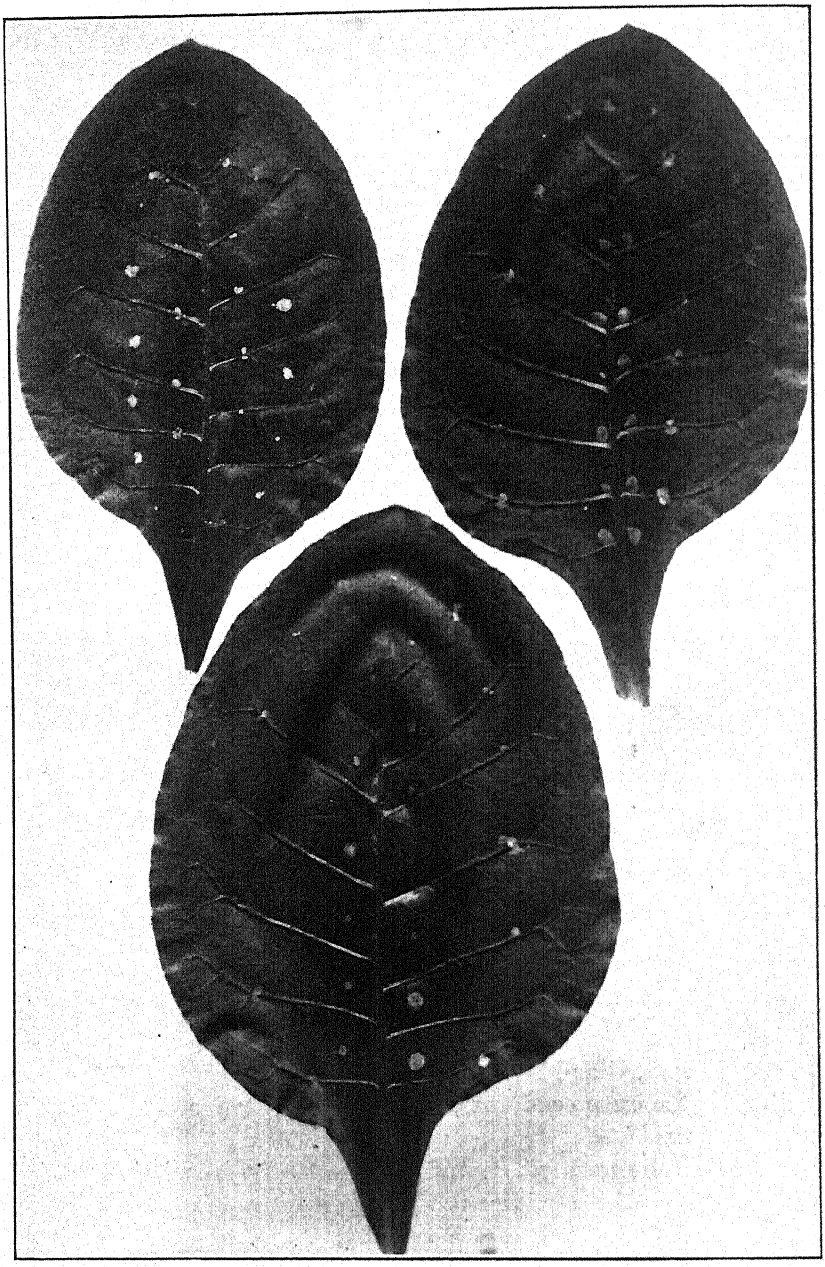


FIG. 2. Results of inoculations with bacterial smear of **Phytomonas polycolor**, n. sp., applied without injuring the surface, on several parts of the leaves. About ½ natural size.

TABLE 1.—*Results of inoculations of upper, lower, and middle leaves of different varieties of tobacco with the bacterial leaf-disease organism*

Variety name	Age of suscept	Method of inoculation	Leaves inoculated	Number of inoculations	Per cent infection after		Reisolation
					4 days	14 days	
Tall Zimmer	3 mo. 23 da.	Culture in beef bouillon	Upper	180	17	17	+
" "	" "	Inoculation through punctures	Middle	180	25	25	+
" "	" "	" "	Lower	180	50	50	+
" "	" "	" "	Upper	60	0	0	−
" "	" "	" "	Middle	60	5	5	+
" "	" "	" "	Lower	60	20	20	+
Baker Sumatra	3 mo.	Bacterial smear	Upper	123	43	46	+
" "	"	" "	Lower	462	68	71	+
P. I. 9352 SP. No. 3	4 mo.	" "	Upper	40	48	48	+
"	"	" "	Middle	40	62	62	+
"	"	" "	Lower	40	100	100	+
N. T. Havanensis	"	" "	Upper	20	20	20	+
" "	"	" "	Middle	20	30	30	+
" "	"	" "	Lower	20	50	50	+

When the same method of inoculation was used under field conditions the amount of infection was comparatively less and showed up more slowly, obviously because of the less favorable environmental factors. What these favorable factors are, however, is not well understood.

Several inoculations made by spraying the suscept with a water suspension of the bacteria gave a few positive results. When cultures in beef bouillon were used as inoculum comparatively few infections were obtained.

A difference was observed in the appearance of the spots produced by the application of a bacterial smear to punctures and those obtained by the application of the smear to the uninjured surface. The disease was readily produced by both methods, but when the smear was applied to the punctures the resulting spot occasionally developed a yellow halo around the lesion. This halo was especially noticeable during the early stage of the infection, but disappeared with age, when the spots became white or sometimes brown. When the latter method was employed the spots were invariably opaque. Table 1 summarizes the results of these inoculations.

A very marked difference in susceptibility among the lower, middle, and upper leaves can be noticed. The lower leaves are the most susceptible, showing in some cases 100 per cent infection; the middle ones ranked second; and the youngest were more rarely infected. Leaves that have been allowed to become mature or overmature are readily infected. Nearly all parts were used for inoculation, but the infection showed much more readily when the inoculations were made between the leaf veins. When the inoculum was placed exactly on the veins, the infection appeared in some cases at both sides, as if the pathogen were unable to infect the veins directly. In other cases, spots developed as on other points on the leaves.

EVIDENCES OF VARIETAL SUSCEPTIBILITY

An examination of the results of the inoculations shows a different degree of infection on each of the four varieties of tobacco employed in the study. Considering only the upper and lower leaf infections, it can be seen that the Tall Zimmer and N. T. Havanensis showed an average of 33.5 per cent and 37.5 per cent, respectively, while Baker Sumatra showed 61.40 per cent and P. I. 9352 S. P. No. 3 showed 73.75 per cent. The two latter varieties had about twice the percentage of infection as the two former ones. The varietal differences in leaf texture may be correlated with degrees of susceptibility. The Sumatra varieties are wrapper types and possess comparatively thinner leaves than the other varieties when grown under suitable condition. Further studies along this line are, however, necessary before a definite conclusion can be made.

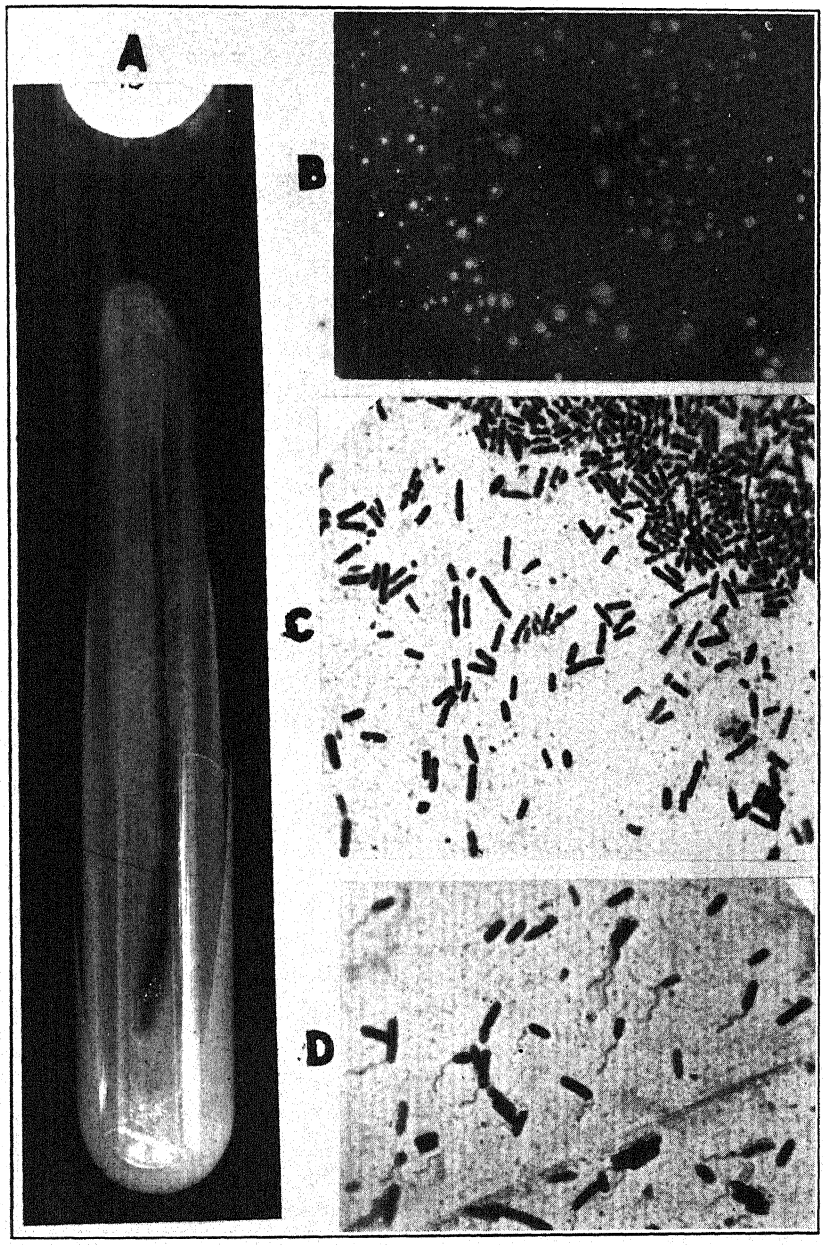


FIG. 3. A. Streak culture on potato-dextrose agar showing the filiform growth widening at the bottom. B. Submerged and surface colonies on potato-dextrose agar. Natural size. C. Bacteria, single and in chains, stained with carbol fuchsin. ×1560. D. Bacteria showing flagella, stained with Plimmer and Paine's stain. ×1560.

THE PATHOGEN

Morphology. The organism is a motile rod with rounded ends; single or in pairs; mono- or lophotrichous (Fig. 3, D). Measurements of stained mounts obtained from 24-hour-old culture on potato-dextrose agar, + 1, ranged from 1.19 to 4.12 μ in length by 1.06 to 1.33 μ in width. The average size is 2.68 by 1.27 μ. The organism is easily stained with carbol fuchsin, methylene blue, aniline gentian violet, and aqueous gentian violet. Plimmer and Paine's stain (6) for flagella gives very good results with 27-hour-old cultures on potato-dextrose agar, + 1, at temperatures 18° to 20° C. One to two flagella, usually one, are observed. They are spiral-like and about two to three times longer than the body of the organism (Fig. 3, C and D). The organism is gram-negative and is not acid-fast.

Cultural characteristics.—Potato-glucose agar, + 1. The organism grows rapidly on this medium. In poured plates the colonies appear on the second day. They are colorless at first, but begin to turn yellow on the third day. In shape they are convex, glistening, with entire margin. Submerged colonies are lenticular and small (Fig. 3, B). On long standing, surface colonies become olive brown. Streak cultures are grayish white to Primuline Yellow[3] at room temperatures, 25° to 30° C. (Fig. 3, A). A yellowish growth is produced at the bottom of some of the cultures. Some of these yellow growths on more than year-old cultures were still viable and pathogenic, while others four months old did not grow at all. Why there was such a difference is not well understood and no attempt was made to study the underlying causes.

In the course of continuous culturing of the organism, 116-C31, three variants developed in some of the stock cultures on potato-glucose agar, and on potato plugs more than a year old. At first it was thought that these seemingly extraneous growths of the cultures were contaminations. Subsequent morphological, physiological, and biological studies, however, showed that none of them fell under this category. The first, type 116g-C31, is characterized by a bluish green color which may or may not be imparted to the medium. Colonies develop green granular pigments at the center in three days under room condition. The green color disappears with age and some yellowish growth develops on the surface. The culture, however, remains viable for a long time. The second subtype (116p-C31) is characterized by the production of pink lavender turning to purple with age on lead-acetate agar. The third subtype, labeled 116w-C31, is a rapid-growing, grayish white organism having a filiform growth on a slant of potato-dextrose agar, + 1, and living only for a short time.

[3] The color names beginning with capitals are those of Ridgway's Color Standards and Nomenclature. Washington, 1912.

Any of these subtypes were readily obtained from colonies either from the yellow type (cul. 116-C31), from the subtype colony with green centers (116g-C31), or from subtype pink lavender colony (116p-C31) on poured plates of suitable media. Although no extensive detailed study of this behavior of the organism other than on solid media, broth, and sugar solutions was made, a certain degree of biological and physiological differences was noted. The subtype 116g-C31, for instance, seemed to remain viable longer in culture under ordinary room conditions than all other cultures. Some variability in the virulence of the subtypes has also been observed. The whole phenomenon finds explanation in bacterial dissociation so ably discussed by Hadley (3).

Beef-peptone agar. Growth along the streak, flat filiform, slightly widening at the bottom, smooth, glistening, fluorescent on the third day but becoming nonfluorescent within seven days, the medium becoming Marguerite Yellow.

Glycerine agar. On this medium a profuse filiform growth, grayish white, smooth, and glistening is produced. An Opaline Green color is produced and imparted to the medium. On long standing the medium becomes greenish brown and a Sulphur Yellow filiform growth on the surface is produced. The organism remains viable on this medium for some time.

Potato cylinder. The growth spreads out, at first yellow glistening and becoming sepia brown. The organism lives long on this medium. Five months and fourteen days under room temperatures showed viable transfers on potato-glucose agar.

Potato-agar slant. A rather poor, colorless to grayish white, filiform, and slightly raised growth develops after the lapse of one day.

Beef bouillon + 1. Growth is very noticeable after 16 hours but is confined to a few centimeters near the surface, indicating strong aërobism. A fragile, flaky pellicle is present on the surface. Green fluorscence appears in the turbid region. On older cultures a white precipitate settles to the bottom.

Gelatine stab. At 18° to 20° C. no liquefaction is observed until the fourth day; at first crateriform, becoming stratiform on the sixth day; the medium completely liquefies in one to four weeks. At 7° to 9° C., liquefaction is very slow. Under this condition no liquefaction appears until after 23 days. The medium becomes completely liquefied and a whitish-gray precipitate within 3 months settles to the bottom.

Loeffler's blood serum. A sunken furrow-like, filiform growth is very noticeable after 16 hours. Frequently the medium cracks and liquefaction starts on the third day, when a distinct disintegration is noticed, generally accompanied by the breaking down of about two-thirds of the slant.

Litmus milk. Apparent change is observed on the third day when a creamy-white to yellow ring is formed around the glass about the surface of the liquid. The liquid becomes Cinnamon Buff, indicating a gradual reduction of litmus starting from the upper portion and continuing gradually towards the bottom. Reduction is complete in twenty-six days and various color changes occur during the process. Neither curdling nor peptonization is produced but a colorless deposit settles to the bottom. The medium became alkaline.

Starch. Starch is not digested.

Beef-agar slant. Growth scanty and noticeable after 16 hours. Grayish white at first, later turning yellow; much less profuse than on potato-dextrose agar and on potato plug.

Indol. No indol is produced on tryptophane broth. Erlich-Böhme technic (7) was employed in the test.

Nitrite in nitrate-peptone broth. Nitrate was reduced to nitrite in nitrate-peptone broth (Merck's nitrite-free reagent). Harper's prenol-disulphonic-acid method (4) for nitrite detection was employed. Johnson did not obtain nitrate reduction with *Phytomonas mellea.*

Litmus-sugar agars. Glycerine, glucose, lactose, and saccharose agars were used. No litmus reduction was obtained by Johnson (5) in any of these sugars with *Ph. mellea,* while, with the Philippine bacterium, litmus is reduced in glucose agar four days after inoculation. The medium becomes Ox Blood Red from the upper portion, extending gradually towards the bottom. In glycerine, lactose, and saccharose, acid is not produced.

Fermentation. The use of Dunham's 2 per cent peptone water resulted in the production of no gas on 1 per cent solutions of the following carbohydrates: xylose, arabinose, levulose, mannose, galactose, glucose, lactose, maltose, saccharose, raffinose, rhamnose, inulin, glycerine, mannite, sorbite, dulcite, inocite, dextrin, salicin, amygdalin, and adonite. For the detection of acid formation, Andrade's indicator was employed. Strong clouding, denoting the growth of the organism, is observed in all cultures 16 hours after inoculation. Clouding invariably appears near the surface of the liquid associated with various shades of fluorescence. Acid formation is noted in xylose, arabinose, glucose, and mannose.

Hydrogen sulphide. Cultures on basic lead-acetate agar show the production of hydrogen sulphide.

Physiological reaction—Temperature relations. At temperatures 7° to 9° C. the organism grows very slowly, at 20° to 24° the growth is moderately profuse, and at 37° to 39° the growth is most profuse, being 8 mm. wide at the bottom in 16 hours, but no viable transfers were obtained after a short time. Gray spots appear on the strokes and no pigmentation.

Johnson (5) did not obtain any growth with *Phytomonas mellea* at 35° to 36° C.

Resistance to desiccation. Drops of beef-bouillon culture placed on sterilized cover glasses kept in sterilized petri dishes are still viable after six days but show no growth after 12 days.

Viability and virulence. The organism grows well on potato-glucose agar, + 1, and remains alive for a long time. Viable transfers are obtained from some cultures more than one year old. Potato plugs and glycerine agar maintain equally well the viability of the organism with no sign of loss of virulence.

Nomenclature. A comparison of this organism with all other known bacteria causing disease of tobacco and of the symptoms produced shows that it resembles *Phytomonas mellea* more than any of the others. No comparative study of the two organisms was made, as no culture of *Ph. mellea* could be obtained. But a comparison of the characteristics of the Philippine organism with those of *Ph. mellea* as described by Johnson (5) presents very significant divergences which would not logically place the Philippine bacterium in the same species. The symptoms produced, the morphology of the organism, reaction in carbon media, reduction of nitrate into nitrite, and action upon milk and starch are very different from those of *Ph. mellea.*

TABLE 2.—*Characteristic differences between Phytomonas mellea and the Philippine organism*

Phytomonas mellea	*Philippine organism*
1. Brown "rust" is produced	1. White spot is produced
2. Chlorotic halo frequently present	2. Chlorotic halo very rarely present
3. Average size of bacterium, 1.8 by 0.6 μ	3. Average size of organism, 2.68 by 1.27 μ
4. One to seven polar flagella	4. One to three polar flagella
5. Growth on potato-dextrose agar, Honey Yellow to deep orange	5. Growth on potato-dextrose agar, Primuline Yellow, fluorescent
6. On potato cylinder, growth brownish yellow	6. On potato cylinder, growth yellowish to sepia brown; bluish green color exhibited at 20° to 22° C.
7. Starch digested	7. Starch not digested
8. Litmus milk becomes alkaline, coagulated, and slowly peptonized	8. Litmus milk rendered alkaline but not curdled nor peptonized
9. Nitrate in nitrate broth, no reduction into nitrite	9. Nitrate in nitrate-peptone broth reduced into nitrite
10. No acid formed in glucose, lactose, and saccharose broth	10. Acid formed in glucose, mannose, xylose, and arabinose but not in lactose and saccharose; fluorescence exhibited

According to Johnson (5), the species *mellea* is so named because of the development of a honey color in culture. It peptonizes milk, digests starch,

and does not reduce nitrate. The Philippine bacterium does not peptonize milk nor digest starch. It reduces nitrate to nitrites and produces a characteristic green fluorescence of various shades in nearly all cultures, particularly in carbon and broth media. Colonies with green centers also are produced. This suggests at once that the organism belongs to the fluorescent group, the plant pathogens of which Bergey *et al.* (1) include in the genus Phytomonas. Burkholder (2), however, in "The Genus Phytomonas," allocates this group to the genus Pseudomonas Migula to include all distinctly green fluorescent bacteria, based upon their logical and significant natural relationship.

Among this group of bacterial pathogens the species that approach it in similarity, although distinctly not identical to it, are *Phytomonas Tolaasi* (Paine) Bergey *et al.*, parasitic on cultivated mushrooms, *Ph. bowlesia* Lewis and Weston, parasitic on *Bowlesia septentrionalis; Ph. martyniae* (Elliott) Bergey *et al.*, parasitic on *Martynia louisiana;* and *Ph. marginalis* (Brown) Bergey *et al.*, parasitic on lettuce. All these species, however, differ from the one studied in their ability to curdle and peptonize milk. The latter two differ also by fermenting sucrose. *Ph. marginalis* differs further in its inability to produce hydrogen sulphide. There may be other species in the group which present more or less similarity to the Philippine organism, but this could not be ascertained because of the present uncertainty or absence of knowledge of their physiological characters. Another very distinctive characteristic of the organism is its ability to produce intensely green pigments on glycerine agar and a pink lavender color, which turns purple, on lead acetate agar. However, some cultures on lead acetate agar behave as on glycerine agar.

It is thus very clear that the organism can not be identified with any of the previously described members of the green fluorescent group nor with any other known bacterial plant pathogen.

TECHNICAL DESCRIPTION

Phytomonas polycolor, n. sp.[4] Rod-shape type with rounded ends; occurring singly in chains and in pairs; average size 2.68 by 1.27 μ; motile by mono- or lophotrichous, one to three flagella but generally one, about two to three times longer than the body; no spores nor capsules were observed; gram-negative and not acid-fast; readily stained with carbol fuchsin, methylene blue, aniline gentian violet, and aqueous gentian violet; a facultative anaërobe and markedly fluorescent.

[4] Bergey *et al.* limited the generic name Pseudomonas Migula to the nonphytopathogenic fluorescent bacteria. However, if the term is applied to all green fluorescent bacteria, as proposed by Burkholder (2), the name of this organism should be *Pseudomonas polycolor,* n. sp.

Agar colonies, convex with entire transparent peripheral margin; colonies producing green centers develop from old cultures; submerged colonies, small and lenticular; streak growth on potato-glucose agar, abundant, raised, widening at the bottom, glistening, smooth and slightly viscid; green fluorescence in some cultures; in nutrient broth, heavy clouding with fluorescence starts from the top, producing delicate flaky membranes; on potato plug, growth spreading, yellowish, becoming sepia brown with age; litmus milk is reduced and rendered alkaline but does not curdle nor peptonize milk; starch is not digested; xylose, arabinose, glucose, and mannose are fermented, producing acid reaction but no gas formation; in other solutions, such as lactose, saccharose, and other sugars used, no fermentation; gelatine and Loeffler's blood serum, liquefied; nitrate reduced to nitrites; no indol production; produces hydrogen sulphide on basic lead acetate agar; optimum temperature, within 25° to 30° C., maximum temperature, 37° to 39° C. The organism is pathogenic on tobacco, *Nicotiana tabacum* L.

CONTROL MEASURES

Seedling infections have always been observed to be carried to the field. Attempts to show seed-borne infection were made by collecting and planting seeds from badly infected plants showing lesions on the capsules. Some of these seeds were planted under bell jars and others under ordinary condition. The disease appeared on the seedlings under both conditions. The organism obtained from these plants was as pathogenic as the original stock culture. Because of this evidence, seed-disinfection tests with silver nitrate, 1-1000, and mercuric chloride, 1-1000, in separate treatments for 10 to 15 minutes, were made as recommended in the case of other bacterial leaf diseases, *i.e.*, wild fire and angular leaf spot. The seeds were rinsed carefully several times with distilled water and dried in the room before sowing. These tests were tried out during two successive years, 1925 and 1926. The former disinfectant gave more encouraging results both in the reduction of the disease and less untoward effects upon the rate of germination. It seems, from the results obtained thus far, that silver nitrate would be more suitable than mercuric chloride for tobacco-seed disinfection under Philippine conditions.

SUMMARY

1. A new bacterial leaf disease of tobacco, characterized by white or opaque spots, has been under observation in the Cagayan Valley and on specimens received from Iloilo during a period of more than two years.

2. The nature of the damage is a reduction in the quality and quantity of the crop produced. Seedlings may also be destroyed entirely or ren-

dered unsuitable for transplanting. The exact extent of losses and the distribution of the disease are not well known.

3. The causal organism of the disease is a bacterium hitherto unknown and is named **Phytomonas polycolor**, n. sp. A description of the organism is given.

4. Artificial inoculations have conclusively shown the pathogenicity of the organism and the reproduction of a disease identical to that found in nature.

5. The damage from the disease seems to be reduced by seed disinfection with silver nitrate, 1-1000, for 10 to 15 minutes. This method serves to destroy other seed-borne organisms, *i.e.*, those causing wild fire and angular leaf spot.

COÖPERATIVE PLANT PATHOLOGY,
BUREAUS OF AGRICULTURE AND SCIENCE,
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INHERITANCE OF IMMUNITY FROM FLAX RUST[1]

A. W. HENRY[2]

INTRODUCTION

The inheritance of many of the morphological characters of the flax plant has been carefully treated by Tammes (7), Davin and Searle (2), and others. Few studies, however, have been reported on the genetics of disease resistance in flax. Tisdale (8) investigated wilt caused by *Fusarium lini* Bolley and concluded that resistance to this disease was determined by multiple factors.

Results are presented in the present paper of studies on the inheritance of immunity from flax rust, caused by *Melampsora lini* var. *liniperda* (Pers.) Lév., made during the years 1923 to 1926. A brief report is also given of progress made during this period in breeding improved rust-immune varieties of seed and fiber flax.

Most of the varieties now grown in North America are susceptible to rust. Some of the wilt-resistant seed flaxes now in use are very susceptible to rust. Since this disease occasionally causes losses as high as ten per cent in yield of seed, it is desirable that new varieties should be immune from rust as well as resistant to wilt. Rust also has been introduced into the fiber-flax centers of North America. The disease is especially important to fiber-flax production since the stems may be rendered worthless for fiber purposes if severely attacked. The introduction of immune varieties would remove this danger. Having found several varieties of seed flax which are immune from rust (4), it was thought possible to transfer this quality into taller varieties suitable for fiber purposes.

PARENTAL MATERIAL

The following rust-immune parents were used in these studies: Argentine selection, Ottawa 770B, and Bombay. Several immune parents were used, as each possessed certain valuable characters in addition to immunity from rust. Moreover, owing to the possibility of forms of rust becoming prevalent that would attack one of these varieties, it was thought advisable to use several immune parents in the crosses.

[1] Contribution from the Department of Plant Pathology, Minnesota Agricultural Experiment Station, St. Paul, Minn. Cooperative investigations between the Minnesota Agricultural Experiment Station and the offices of Cereal Crops and Diseases and Fiber Plants of the U. S. Department of Agriculture.

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All three varieties have been thoroughly tested with different collections of flax rust and have remained immune in all tests made by the writer and in all tests known to him. A few susceptible plants have been found in Argentine selection and Bombay, but these quite evidently were mechanical mixtures or the result of natural crossing, as the bulk of the plants proved immune. Tests have been made with several American collections of flax rust from both the United States and Canada, as has been previously reported (3). Argentine selection and Ottawa 770B, together with several susceptible varieties, such as Winona and Saginaw, have been tested by Hiratsuka (6) in Japan, with the same results as ours. Argentine selection and Ottawa 770B were also immune from the Japanese forms of this rust. During the summer of 1927, while in Europe, the writer inoculated these three varieties with a collection of flax rust kindly supplied by Dr. Tine Tammes from her flax-breeding garden at the University of Groningen, Groningen, The Netherlands, and all three remained immune. Their choice as parents from the standpoint of their reaction to rust seems, therefore, to have been a fortunate one.

The above parents were obtained originally from the following sources: Argentine selection, from commercial Argentine flax obtained from the Pittsburgh Plate Glass Company of Red Wing, Minnesota, and the original selections made by Dr. H. D. Barker, formerly of the Minnesota Agricultural Experiment Station and U. S. Department of Agriculture; Ottawa 770B, from Mr. R. L. Davis, formerly in charge of fiber-flax breeding for the U. S. Department of Agriculture at East Lansing, Michigan, who had previously received it from the Dominion of Canada Department of Agriculture; and Bombay C. I. 42, kindly provided by J. C. Brinsmade, Jr., of the Northern Great Plains Field Station of the U. S. Department of Agriculture at Mandan, North Dakota.

Argentine selection, in addition to being immune from rust, is also highly wilt-resistant. This has been shown by several years tests on "sick" soil at University Farm, St. Paul. It is a large, brown-seed, late-maturing type of medium height, with large blue flowers. The stems, however, are too short and coarse and branch too freely to be of value for fiber purposes. Numerous selections of this type of flax, obtained from different commercial lots of Argentine flax, have thus far been immune from rust (3).

Ottawa 770B is a medium-size, yellow-seed variety with small white crimpled flowers. It matures too late and yields too little seed to be used as a seed flax. Although somewhat taller than Argentine selection, it is not tall enough nor fine enough in the stem to make a good fiber variety. Bombay is a medium-size, brown-seed variety having small blue flowers. It is a short, early-maturing type. It is too short, however, to become of impor-

tance as a seed flax in the seed-flax areas of the United States and is naturally also unsuitable as a fiber variety.

The susceptible varieties chiefly used in these studies were Saginaw and Winona. Saginaw is a very tall variety of fiber flax developed by the Office of Fiber Plants of the U. S. Department of Agriculture. It has small brown seeds, small bolls, and small blue flowers. It has long, fine stems but produces very little seed. It is quite susceptible to rust, though it usually does not develop as heavy infections in the later stages as does Winona. In maturity it is relatively early for so tall a variety. Winona is a medium-early variety of seed flax developed at the University of Minnesota by the Departments of Plant Pathology and Plant Breeding. It was selected particularly for its excellence in wilt resistance, from Blue Dutch Minn. 175 (1). It has small, brown seed and small, dark blue flowers. Though one of the best wilt-resistant seed flaxes, it is very susceptible to rust. Two other varieties of seed flax similar to Winona, namely, Blue Blossom Dutch and Chippewa, were used as parents in some of the crosses. The former was obtained from the U. S. Department of Agriculture, while the latter is another wilt-resistant selection produced by the Minnesota Agricultural Experiment Station, being a selection from Primost, Minn. 25 (1).

METHODS

Most of the crosses were made in the field at University Farm, St. Paul, Minnesota, during the summer of 1923.[3] For the most part, the emasculated flowers were pollinated immediately and covered with cigarette tubes fastened with small paper clips and placed close to the flower pedicels and parallel to them.

Argentine selection was crossed with Saginaw, Winona, and Chippewa, but the inheritance of immunity from rust was not studied in detail in the crosses with the two latter varieties. In most of the Argentine selection by Saginaw crosses, Argentine selection was used as the female parent, since it sets seed better than Saginaw and since its large flowers are easier to manipulate. However, the reciprocal cross also was made. Ottawa 770B, as the male parent, was crossed with Saginaw and Winona. Bombay was used as the male parent in crosses with Winona, Blue Blossom Dutch, and Saginaw. Detailed inheritance studies were made only on the first two of these crosses. Some of the data were obtained from greenhouse inoculations; for instance, the F_1 of the Argentine selection by Saginaw crosses. This was grown in the greenhouse during the winter of 1923–24. Artificial

[3] A few crosses were made by H. D. Barker in the greenhouse in the winter of 1922–23 between Argentine selection and Saginaw. Five hybrid seeds were obtained from these crosses.

light was used at night in order to hasten the maturity of the plants. They were first inoculated with rust in the seedling stage by applying urediniospores to the cotyledons and leaves by means of a flat inoculating needle. They were inoculated a second and a third time by spraying with a suspension of urediniospores in water as well as by dusting with dry urediniospores from infected plants. Part of the F_2 of this cross as well as plants from a back-cross of the F_1 on Saginaw were also inoculated in the greenhouse and treated in a manner similar to that employed with the F_1. Parental plants also were inoculated to serve as checks with each set of inoculations.

Data on the rust reaction of the remainder of the F_2 and of the F_3 of the Saginaw × Argentine-selection cross, together with all generations of the crosses in which Bombay and Ottawa 770B were the immune parents, were obtained in the field. The rust nurseries where this material was tested were sown on peat soil at Coon Creek, Anoka County, and Clearspring, Hennepin County, Minnesota.[4]

It was found advisable to use the peat bogs for these nurseries, since epiphytotics were readily obtained there each year, whereas on higher land they could not always be obtained with certainty. The nurseries were artificially inoculated in order to insure early and uniformly heavy infections. The variety Winona, especially susceptible to rust, was largely grown for borders and intervening rows in the nurseries on which to increase the rust. Plants heavily infected with urediniospores were brought from the greenhouse, and the rust was dusted or sprayed on the plants in the field until a uniform epiphytotic was obtained. Old teliospore-bearing straw also was spread over the nurseries in the spring or early summer in some cases.

RUST REACTION OF THE F_1

F_1 plants of all crosses were inoculated with rust either in the field or in the greenhouse. The result was the same in every test. The F_1 plants were all immune, like their immune parents Argentine selection, Ottawa 770B, and Bombay. The susceptible parents inoculated at the same time were invariably infected. Immunity from rust in these crosses evidently is completely dominant over susceptibility.

SEGREGATION IN THE F_2

The F_2 plants were classified into two categories with respect to rust reaction, namely, immune and susceptible. Those showing no trace of rust were placed in the immune class, while those that developed rust pustules were classed as susceptible.

[4] Thanks are due to the Division of Soils, University of Minnesota, for providing the land at Coon Creek, and to the Division of Agricultural Engineering, University of Minnesota, for that at Clearspring, for these experiments.

The results of inoculating the F_2 plants of the Argentine selection × Saginaw cross and its reciprocal are given in table 1.

TABLE 1.—*Rust reaction of the F_2 of the Argentine selection × Saginaw and Saginaw × Argentine selection crosses*

Cross	Place tested	Number of plants observed		Number of plants expected[a]		Deviation / P. E.
		Immune	Susceptible	Immune	Susceptible	
A × S	Greenhouse	209	17	211.875	14.125	1.17
A × S	Field	319	19	316.875	21.125	0.71
S × A	Greenhouse	107	9	108.750	7.250	0.99
Total	Greenhouse and field	635	45	637.500	42.500	0.59

[a] Assuming a segregation of 15 immune plants to 1 susceptible plant.

Assuming a 15:1 segregation, the deviation of the observed from the expected is in no case greater than might occur from chance alone. It will be noted that the results were the same when Argentine selection was used as the male parent as when employed as the female parent.

In those crosses in which Ottawa 770B was used as the immune parent, notes were taken on the flower color of the F_2 plants as well as on their rust reaction, since Ottawa 770B has white flowers while the susceptible parents have blue flowers. The F_2 results of the Saginaw × Ottawa 770B are shown in table 2. It will be noted that the observed numbers fit a dihybrid ratio very well. The inheritance of rust reaction in this cross is evidently simpler than in the previous cross. Immunity from rust here is

TABLE 2.—*Segregation for rust reaction and flower color of the F_2 of the Saginaw × Ottawa 770B cross*

Cross	Number of blue-blossom plants		Number of white-blossom plants	
	Immune	Susceptible	Immune	Susceptible
Saginaw × Ottawa 770B observed	441	145	142	40
" " " expected				
9:3:3:1	432	144	144	48

$\chi^2 = 1.556$ $\quad p = 0.6741$

apparently determined by a single factor, as is also flower color. Rust reaction and flower color in this cross evidently are inherited independently

of each other. All white-flower segregates invariably bore yellow seed and crimpled petals, like Ottawa 770B. There is a deficiency in numbers of white plants. As might be expected, this is more pronounced when the numbers are based on the F_3 families. Results for the Winona × Ottawa 770B cross are given in table 3. Tammes (7) obtained comparable results in studying the inheritance of flower color in similar crosses. She attributes her results to a factor C^1, "this factor having a semi-lethal effect in the absence of B_1, which renders the combination of gametes white with white less viable. In consequence of this there arise capsules as compared with other genotypes averaging fewer seeds, whilst the seed formed has less germinative power."

TABLE 3.—*Segregation for rust reaction and flower color in the F_2 of the Winona × Ottawa 770B cross, based on the behavior of the F_3 families*

Cross	Number of blue-blossom plants		Number of white-blossom plants	
	Immune	Susceptible	Immune	Susceptible
Winona × Ottawa 770B observed	182	53	36	11
" " " expected 9:3:3:1	158.625	52.875	52.875	17.625

$\chi^2 = 11.32$ $p = 0.0112$

In the above table all families showing segregation for rust reaction were considered to have come from heterozygous F_2 plants and are therefore classed in the immune phenotype of that generation. All families showing segregation for flower color are classed as blue for a similar reason. Due largely to the deficiency of white plants, the deviation of the observed ratio from the theoretical is of the magnitude shown. Such a deviation could be attributed to chance only about once in a hundred times. The segregation for rust reaction alone is in good agreement with an expected 3:1 ratio, there being 218 plants classified as immune and 64 as susceptible, a deviation from the expected of 1.33 times the probable error. The immune variety Bombay was crossed with the susceptible varieties Saginaw, Winona, and Blue Blossom Dutch. The results of the Saginaw crosses are not reported here, as the numbers were too small to be conclusive, though the segregation appeared to be similar to that of the other crosses. The segregation for rust reaction in the F_2 of these crosses is shown in table 4.

The two susceptible parents in these crosses are very similar. They were both heavily infected with rust, as were also the susceptible segregates of the F_2. The latter stood out very sharply from the immune plants and could readily be distinguished at a distance by the abundance of rust on

TABLE 4.—*Rust reaction of the F_2 of Winona × Bombay and Blue Blossom Dutch × Bombay*

Cross	Number of plants observed		Number of plants expected		Deviation / P. E.
	Immune	Susceptible	Immune	Susceptible	
Winona × Bombay	474	153	470.25	156.75	0.51
Blue Blossom Dutch × Bombay	251	92	257.25	85.75	1.15

them. The Winona plants and the susceptible segregates of the Winona × Bombay cross were most heavily rusted, so severely, in fact, that a considerable number of the susceptible segregates were killed before they had formed seed. The ratios of the immune to susceptible plants in both crosses are in close agreement with a 3 : 1 expectation.

Back Cross. The F_1 of the Saginaw × Argentine cross was back-crossed on Saginaw, the susceptible parent. These crosses were made in the greenhouse and the seedlings from the crossed seed were inoculated with rust three times. At the same time F_2 seedlings, derived from seed of these same F_1 plants used in the back crosses, were inoculated in a similar manner and under the same conditions. The results are shown in table 5.

TABLE 5.—*Rust reaction of Saginaw × (Saginaw × Argentine) selection seedlings compared with that of F_2 seedlings of the Saginaw × Argentine selection cross*

Cross	Number of plants observed		Number of plants expected		Deviation / P. E.
	Immune	Susceptible	Immune	Susceptible	
S × (S × A)	51	18	51.75(3)	17.25(1)	0.31
S × A	107	9	108.75(15)	7.25(1)	0.99

It will be seen that the segregation of the back-cross seedlings for rust reaction fits a 3 : 1 expectation very well, while the segregation of the F_2 seedlings under the same conditions is in good agreement with a 15 : 1 expectation. These results therefore support the hypothesis that there are two duplicate factors determining rust reaction in this cross.

RUST REACTION OF THE F_3

F_3 families of the Argentine selection × Saginaw cross were tested in the field. Assuming that rust reaction in this cross is determined by two duplicate factors, a ratio of 7 : 8 : 1 would be expected in the F_3. Seven out of 16 families should breed true for immunity from rust, 8 families should segregate, 4 in a ratio of 3 immune plants to 1 susceptible, and 4 in

a ratio of 15 immune plants to 1 susceptible plant, and 1 family out of 16 should breed true for susceptibility to rust. Among the segregating families observed there were some which were clearly segregating in a 3 : 1 ratio and others which were clearly segregating in a 15 : 1 ratio. Several of these families contained between 50 and 100 plants. There were other families containing fewer plants in which the ratio of immune to susceptible plants was such that they could not be definitely assigned to either the 3 : 1 or the 15 : 1 group. Consequently, in table 6 all of the segregating families

TABLE 6.—*Rust reaction of F_3 families of the Argentine selection × Saginaw cross*

	Number of families breeding true for immunity	Number of segregating families	Number of families breeding true for susceptibility
Observed	98	88	13
Expected (7 : 8 : 1)	87.1	99.5	12.4

$x^2 = 2.73$ $p = 0.2622$

are placed in one class. The chances that such a deviation from the expected as is shown in table 6 is merely due to random sampling are about one in four. The immune class is larger and the segregating class smaller than expected. It is quite probable that several of the families placed in the immune class should go in the segregating class. For instance, in a small family segregating in a ratio of 15 immune to 1 susceptible the susceptible plants might be overlooked, as the infections were often confined in this cross to a few pustules per plant.

The F_3 families of the other crosses studied were grown at University Farm, St. Paul, the following year (1926). In order to save time and expense, an effort was made to obtain an epiphytotic of flax rust there, but the season was very unfavorable for rust development. When by midsummer it was realized that it would not be possible to obtain a uniform infection, as many as possible of the F_3 families were resown with reserve seed on a peat bog at Clearspring, Minnesota. The plants were too late to form seed, but a fairly good epiphytotic of rust was obtained.

F_3 families of two of the Ottawa 770B crosses were grown, namely, Saginaw × Ottawa 770B and Winona × Ottawa 770B. The infection on the former was not very heavy, and, consequently, the results are not sufficiently reliable to report. As has been previously mentioned, Saginaw, the susceptible parent in this cross, often develops only a few pustules of rust, and, if the plants are not subjected to a very heavy and uniform epiphytotic, some of them may be classed as immune. Under the conditions of the test, only about 90 per cent of the Saginaw plants became rusted. In the

case of the much more susceptible variety Winona, however, 100 per cent of the plants developed rust. The results of the Winona × Ottawa 770B cross are shown in table 7. In a few of the families counted as susceptible,

TABLE 7.—*Rust reaction of F_3 families of the Winona × Ottawa 770B cross*

Cross	Number of families			Total
	Immune	Segregating	Susceptible	
Winona × Ottawa 770B observed.....	80	152	66	298
" × " " expected.....	74.5	149	74.5	298

$x^2 = 1.44$ $p = 0.50$

there were a few plants which did not become rusted. It is probable that some of these were natural hybrids with immune plants. Immunity being dominant, they would, of course, be free from rust. None were counted susceptible, however, which did not show more than 50 per cent of the plants rusted. There would be one chance in every two of a deviation, as great as that shown, of the observed from the theoretical being due to random sampling.

In the Winona × Bombay cross, the observed F_3 ratio, as shown in table 8, departs considerably from the theoretical. There is only about one chance in 100 that such a departure from the expected is attributable to chance. Evidently, a disturbing influence has affected this ratio. The susceptible class is only about half as large as it should be. As was pointed out in connection with the F_2, a considerable number of the susceptible segregates were so severely rusted that they failed to form seed. This largely accounts for the small susceptible class of the F_3. In the Blue Blossom

TABLE 8.—*Rust reaction of F_3 families of the crosses Winona × Bombay and Blue Blossom Dutch × Bombay*

Cross	Number of families			Total	x^2	p
	Immune	Segregating	Susceptible			
Winona × Bombay observed	30	67	14	111		
Winona × Bombay expected 1: 2: 1.....	27.75	55.50	27.75	111	9.37	0.01
Blue Blossom Dutch × Bombay observed	34	60	35	129		
Blue Blossom Dutch × Bombay expected 1: 2: 1.....	32.25	64.50	32.25	129	0.63	0.8

TABLE 9.—*Ratios of immune to susceptible plants in 25 segregating families in each of the 3 crosses, Winona × Ottawa 770B, Winona × Bombay, and Blue Blossom Dutch × Bombay*

No. of family	Winona × Ottawa 770B		No. of family	Winona × Bombay		No. of family	Blue Blossom Dutch × Bombay	
	No. of immune plants	No. of susceptible plants		No. of immune plants	No. of susceptible plants		No. of immune plants	No. of susceptible plants
1	19	9	200	20	8	160	13	4
3	25	8	201	33	9	161	19	9
4	23	8	203	22	6	163	21	5
5	17	1	205	22	6	172	16	11
8	25	7	208	19	14	177	11	8
12	21	2	209	25	11	179	9	4
13	23	2	210	20	15	180	13	4
14	12	5	214	16	7	183	2	2
16	14	3	215	25	7	185	15	11
17	10	1	218	15	3	187	21	3
18	12	2	219	13	2	189	9	4
19	7	1	220	22	6	192	21	3
22	12	4	223	20	5	193	19	11
23	7	2	224	22	2	198	20	3
24	11	5	225	15	11	199	23	4
25	24	4	226	15	13	214	8	6
26	24	10	228	21	7	215	17	9
27	22	17	229	20	3	216	15	4
29	31	6	230	17	3	217	21	5
33	5	2	233	21	9	218	5	2
36	20	8	236	19	5	219	3	1
37	11	9	237	18	7	221	6	4
40	24	7	240	24	12	223	19	7
45	21	16	241	9	8	224	20	11
46	14	2	243	22	7	225	21	7
Totals (obs.)	434	141		495	186		367	142
Expected 3:1	431.25	143.75		510.75	170.25		381.75	127.25
Deviation / P. E.	0.39			2.07			2.24	

Dutch × Bombay cross, x^2 is less than one and, consequently, p indicates a close agreement between the observed and theoretical ratios.

Assuming that there is but a single-factor difference between immunity and susceptibility in the Ottawa 770B and Bombay crosses and a complete dominance of immunity over susceptibility, the segregating families of the F_3 should give 3 (immune) : 1 (susceptible) ratios. The observed ratios fulfill this expectation, as is shown in table 9. The deviation from the expected is in no case greater than three times the probable error.

BREEDING WORK

The parents used in the crosses discussed possess, as has been pointed out, many valuable characteristics, included among them immunity from rust and a high degree of wilt resistance, though each of them lacks one or more desired characters.

As the primary object of the investigations is to develop varieties of seed and fiber flax possessing not only immunity from rust but wilt resistance and other desirable characters as well, numerous selections have been made with these points in mind.

The results of the inheritance studies have furnished a valuable guide for the selection work. By selecting from F_3 families breeding true for immunity from rust and flower color, one can be assured that the progenies of these selections will also breed true. However, because of the occurrence of natural crossing (5), it is advisable to protect the selected plants from foreign pollen. Selections were, of course, also made from segregating families which appeared promising. In making the original selections, particular attention was given to earliness, height, diameter of stem, size and color of seed, size and color of flowers, degree of branching, and yield of seed, as well as to immunity from rust. Types unsuitable for fiber purposes might well prove valuable for seed purposes. The plan for the further testing of the selections calls for a wilt-resistant test on soil infested with the wilt organism, finally a yield and oil test on those surviving selections suitable for seed flaxes, and a yield and quality test especially of the stems and their fiber content of the surviving tall selections suitable for fiber flaxes.

It was evident from a study of the F_3 families of the Ottawa 770B and Bombay crosses and of the F_3 and F_4 families of the Argentine selection crosses that immunity from rust could be combined with many desirable qualities not possessed by the immune parents. Thus, in figure 1, A, (right) we see it combined with greater height in an F_3 family of the Saginaw × Ottawa 770B cross. This family bred true for immunity from rust and for white flowers and yellow seed. The immune parent, Ottawa 770B, is shown at the left. The white-flower selections from the Ottawa

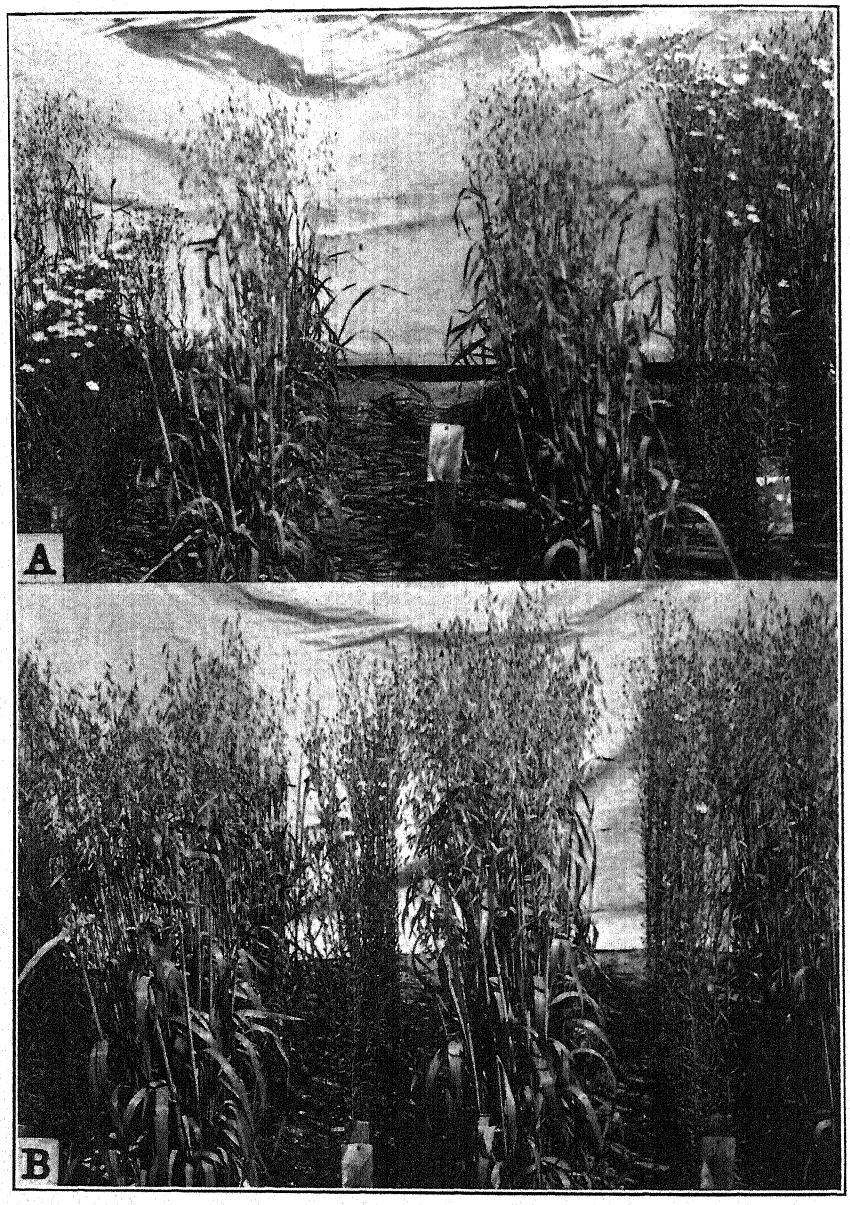


FIG. 1. A. Left; Ottawa 770B, one of the rust-immune parents. Right; Tall, white-flower F_3 family of the cross Saginaw × Ottawa 770B. This family bred true for immunity from rust, flower, and seed color. B. Two tall blue-flower F_3 families of the Saginaw × Ottawa 770B cross.

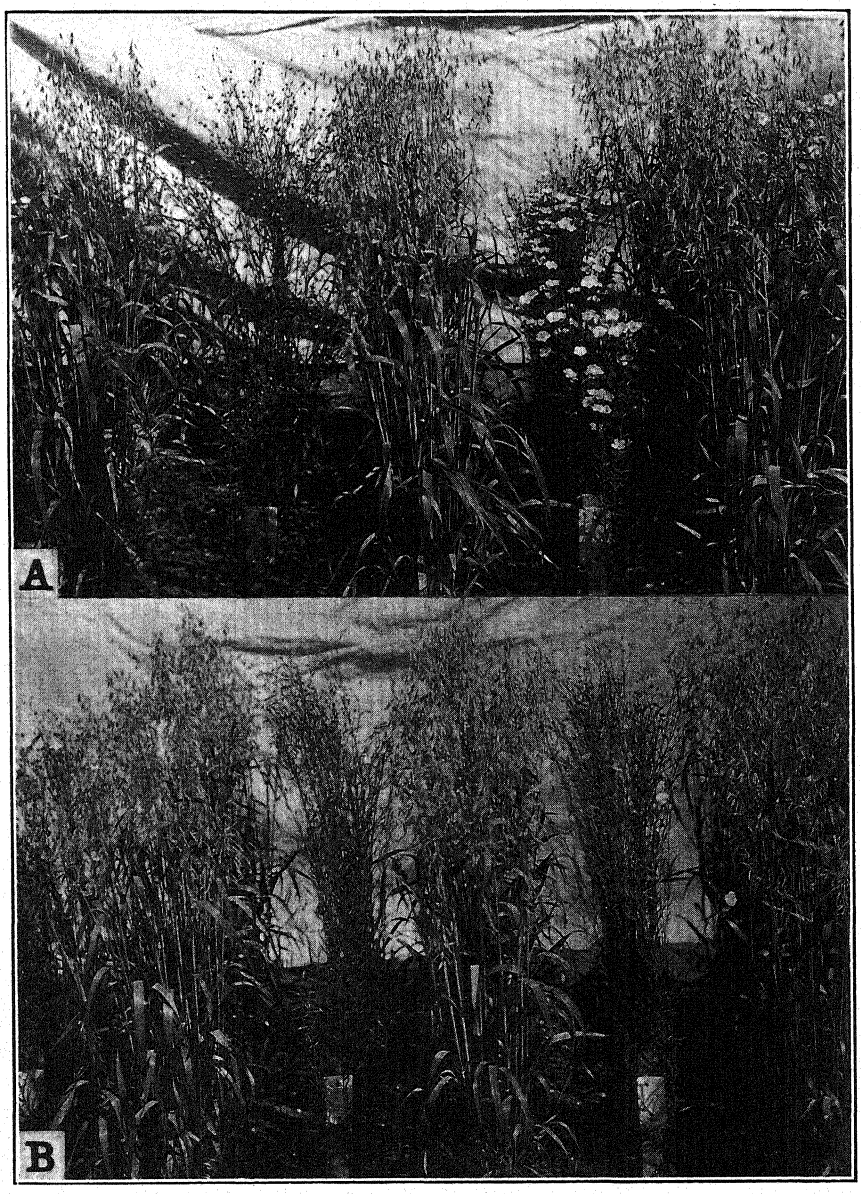


FIG. 2. A. Left; Saginaw fiber flax, a susceptible parent. Right; Argentine selection, immune from rust and resistant to wilt. B. Two tall F_4 families of the Saginaw × Argentine selection cross. These resemble the susceptible parent in height and earliness and the immune parent in freedom from rust.

770B selections, because of low yield, may not be suitable for seed flaxes but may be valuable as fiber flaxes provided suitable yield and quality of fiber can be obtained in them. Their distinctive flowers would be useful in keeping them pure. In figure 1, B, are shown two of the taller blue-flower F_3 families from this same cross. Some of these bred true for immunity from rust and for blue flowers and brown seed; moreover, they were much taller and earlier than Ottawa 770B, the immune parent. Promising selections of the seed flax type were obtained from the Winona × Ottawa 770B cross as well as from the Argentine selection, and a few from the Bombay crosses. Figure 2 shows the great difference in earliness between Saginaw and Argentine selection, while figure 2, B, shows F_4 selections from a cross between these two varieties which have the height and earliness of the susceptible parent and the freedom from rust of the immune parent.

Although the writer did not have an opportunity to test the relative wilt resistance of many of the selections, it is very probable that a number of them will prove wilt-resistant as well as immune from rust. The parents Argentine selection and Winona both possess wilt resistance to a high degree, and the fact that the former also is immune from rust indicates that the two qualities can be combined in the one variety. The development of improved varieties of seed and fiber flax combining resistance to these two major diseases will constitute an important step. Further research should aim to introduce resistance to still other diseases, such as pasmo, browning, and anthracnose, into new varieties.

SUMMARY

1. Argentine selection, Ottawa 770B, and Bombay, three varieties immune from flax rust, were used as parents in the investigations reported.

2. All three varieties have been inoculated by the writer with several North American collections of flax rust and with one European collection. The first two varieties also have been tested by Hiratsuka in Japan. They have remained immune in all tests.

3. The immune varieties were crossed with susceptible varieties of fiber and seed flax. The principal susceptible parents used were Saginaw and Winona. The latter is more susceptible than the former, particularly in the later stages.

4. Immunity from rust proved completely dominant in all crosses. The F_1 plants were invariably immune.

5. Evidence was obtained in the case of the Argentine selection × Saginaw cross which indicates that immunity from rust depends on two duplicate factors for its expression.

6. In the crosses in which Ottawa 770B and Bombay were used as immune parents, the results of inoculations indicate but a single factor difference between immunity and susceptibility.

7. Immunity from rust was apparently inherited independently of flower and seed color in crosses of Ottawa 770B on Winona and Saginaw.

8. Numerous selections were made from F_3 and F_4 families of the various crosses. Many of these appeared promising, some for fiber purposes and others for seed purposes.

9. Immunity from rust was combined in these selections with various characters not possessed by the immune parents, such as greater height, greater earliness, fewer branches, and difference in color and size of flowers and seeds.

10. Improved selections combining immunity from rust and wilt resistance should also be obtainable from the crosses made.

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GROWTH OF PLANT PATHOGENIC BACTERIA IN SYNTHETIC CULTURE MEDIA WITH SPECIAL REFERENCE TO PHYTOMONAS MALVACEARA

I. M. LEWIS

INTRODUCTION

Although synthetic culture media have been extensively employed in studies of bacterial nutrition and for the purpose of differentiating species or strains in other groups, there appears to have been but few similar studies made on the species pathogenic to plants. Following the lead of Erwin F. Smith nearly all investigators have made use of the synthetic media of Uschinsky (8), Fermi (8), and Cohn (8) in the study and description of new species.

It is obvious that such a complex medium as that of Uschinsky is not well suited to the purpose of determining precisely which carbon or nitrogen compounds are suitable for the growth of an organism. Ability to grow in either Fermi's or Cohn's solution denotes utilization of ammonia as a source of nitrogen, but failure to grow does not definitely prove inability to assimilate ammonia, since either glycerin or tartaric acid may be unsuited as a source of carbon. The accurate determination of nutritive requirements is possible only when carbon and nitrogen compounds are so combined as to afford but a single source of each in the culture medium. When a variety of such media is employed it is possible definitely to determine carbon and nitrogen requirements.

Use of such media has often been made in the study of other groups, and our knowledge of bacterial nutrition has been markedly advanced by such studies. In addition valuable means of differentiating closely related species or strains based on utilization of a single source of nitrogen or carbon have been discovered. Koser (5, 6) has shown that differentiation within the colon group may be accomplished by employing synthetic media containing citrate as the sole source of carbon or uric acid as the source of nitrogen. Similarly Simmons (7) and others have shown differentiation within the paratyphoid group. Recently, Frazier and Rupp (3) have suggested the possibility of grouping proteolytic bacteria by growth in media containing ammonia or urea with the addition of sugars or organic acids.

Braun and his collaborators have emphasized the necessity of certain refinements in technique not generally practiced by earlier investigators. Braun and Cann-Bronner (1) were apparently the first to recognize the importance of testing the ability of an organism to grow in serial trans-

TABLE 1.—*The growth of plant-pathogenic bacteria in the synthetic media of Uschinsky, Fermi, and Cohn. Compiled from data published by various authors*

Name of species	Medium			Author
	Uschinsky	Fermi	Cohn	
Erwinia amylovora	−[c]		−	Smith
" aroidea	+−[b]			Townsend
" atroseptica	+[a]	+	−	Morse
" carotovora	+			Jones
" nicotiana	+−		−	Smith
" oleracea	+	+		Harrison
" solanisapra	+	+		Harrison
" tracheiphila	+−	+−	+−	Smith
Phytomonas alboprecipitans	+	+	−	Rosen
" angulata	+			Fromme and Murray
" apii	−		−	Jagger
" aptata	+	+	−	Brown and Jamison
" atrofaciens	+	+	−	McCulloch
" beticola	+	+	−	Brown
" bowlesia	+	+	+−	Lewis and Watson
" campestris	+−	+−	+−	Smith
" cannae	+	+	+−	Bryan
" citrarifaciens	+	+	+	Lee
" coronafaciens	+	+	+	Elliott
" cucurbitae	+		−	Bryan
" delphinii	+	+	+	Bryan
" dissolvens	+−	−	−	Jones
" erodii	+	+	+−	Lewis
" exitosa	−	−	−	Gardner and Kendrick
" flaccumfaciens	+	+−	+−	Hedges
" gummisudans	+−	+−	+−	McCulloch
" holci	+	+	−	Kendrick
" hyacinthi	+			Smith
" insidiosum	−	−	−	Jones and McCulloch
" lachrymans	+	+	+	Smith and Bryan
" maculicola	+−		+−	Smith
" malvaceara	+		−	Smith
" marginale	+	−	−	Brown
" marginata	+	+	+	McCulloch
" martyniae	+	+	+	Elliott
" mellea	+	+		Johnson
" michiganensis	−	−	−	Smith
" mori	+		−	Smith
" panici	+	+	+	Elliott
" papulans	+		−	Rose
" pelargoni	+	+	−	Brown
" phaseoli	+−			Smith
" pruni	+			Brown
" puerariae	+	+		Hedges
" rathayi			−	Smith
" savastonoi	+	+	−	Smith
" solanaceara		+−	−	Smith
" stewarti	+	+−	−	Smith
" syringae	+	+	+−	Bryan
" translucens	+	−	−	Jones *et al.*
" trifoliora	+	+	−	Jones *et al.*
" tumefaciens	+−		−	Smith and Townsend
" vasculara	+−		−	Smith
" vignae	+	+	−	Gardner and Kendrick
" viridilivida	+	+	+−	Brown
" viridifaciens	+	+	−	Tisdale and Williamson
" vitians	+	−	−	Brown
" xanthochlora	+		−	Smith

[a] + = "Good growth." [b] +− = "Feeble," "scant," "wanting." [c] − = No growth.

plants in simple synthetic media. Their results show that in some cases an organism may be able to grow when transplanted from complex media but fail to maintain growth in successive transplants in the synthetic medium. This appears to be true particularly in the case of old cultures grown on such media as beef-extract-peptone broth or agar. Since an organism should be able to maintain itself indefinitely in suitable media when transferred at proper intervals it is only necessary to make sufficient serial transplants in order to eliminate this source of error. Braun and Cahn-Bronner (1) consider growth in less than three serial transplants as unreliable. They called attention also to the necessity of affording proper aeration for cultures in such simple media as must be employed. They made use of small flasks containing only sufficient medium to form a shallow layer. Positive results often were obtained by this method when no growth occurred in tubes containing deep layers.

Before beginning the experimental work of the present investigation, it was deemed essential to review the literature of the group thoroughly to determine the present status of our knowledge of the nitrogen and carbon sources utilized by the various species. In so far as it was possible to do so, original descriptions of the species have been consulted. Since data are limited principally to the tests made in the media of Uschinsky, Fermi, and Cohn it seems desirable to tabulate all of the results for convenient reference. (Table 1.) The species names published in the third edition of Bergey's Manual of Determinative Bacteriology have been adopted.

In considering table 1 it must be borne in mind that little or nothing is said as to method of inoculation, time required for growth, number of strains tested, aeration of cultures, or ability to grow in serial transplants. Such expressions as "growth scant," "slight," "feeble," "transient," "doubtful," or "wanting" are frequently met.

Table 1 shows that most of the species are capable of growth in Uschinsky's medium, thus denoting ability to use ammonia or asparagin, or both, as the source of nitrogen, and one or more of the compounds, asparagin, glycerin, or lactate, as the source of carbon. A comparison of the results obtained in the media of Fermi and Cohn shows that Cohn's medium is less satisfactory. Since these media are essentially alike except for the carbon compound, it appears that tartrate is less suitable than glycerin for most species.

More precise data are available for a few species only. Jones (4) tested the growth of *Erwinia carotovora* in a synthetic medium containing ammonia as a sole source of nitrogen and sodium acetate or formate as the only source of carbon. The organism assimilates ammonia and acetate but not formate. Smith (9, p. 290) obtained no growth of *E. tracheiphila* in

asparagin water containing dextrose and mineral salts or in filtered boiled river water containing 1.0 per cent sodium asparaginate, 1.0 per cent dextrose, and 2.0 per cent glycerin. Similar results were obtained when ammonium lactate or tartrate was substituted for sodium asparaginate. Bryan (2) tested the growth of *Phytomonas delphinii* in solutions of peptone, asparagin, and the ammonium salts of citric, tartaric, and succinic acids. The organism uses ammonia, citric, and succinic acids but not tartaric acid. She also reported growth in Uschinsky's and Fermi's but not in Cohn's medium. Smith (10) studied sixteen strains of *Ph. solanaceara*. He found that both urea and ammonia were suitable sources of nitrogen and that growth occurred with dextrose, sucrose, and sodium salts of citric, malic, tartaric, and succinic acids. There was no growth with lactate, acetate, or butyrate. Higgins found that a species pathogenic to peppers was unable to grow in a medium containing only asparagin. The most complete study of the carbon and nitrogen requirements of a plant-pathogenic organism appears to be that of Honing for *Ph. solanaceara*. The results of this study are fully summarized by Smith (10, pp. 244–254). It is shown that asparagin, glycocoll, potassium nitrate, potassium nitrite, and ammonia may serve as sources of nitrogen in the presence of a suitable source of carbon. Certain carbon compounds were found to be absolutely nonusable in the combinations tested. These included glycogen, starch, lichenin, sodium acetate, and sodium butyrate. The most notable feature of this study was the extreme variability of the several strains in the same medium. Some strains almost invariably failed to grow in media which supported growth of others. Similar results were obtained by Braun (1) and by Koser (5) in other groups, thus emphasizing the necessity of including several strains of a species in studies of this kind.

From the data at hand it is obvious that our knowledge concerning utilization of either carbon or nitrogen compounds by species of plant pathogens is far from complete. It appears that ammonia is suitable for the growth of most species and that variation is to be expected in the utilization of various sources of carbon. Before such tests can be considered as of differential value among closely related species, it must be shown that uniformity prevails among several strains of such species. That such tests might prove to be of such differential value, as has already been shown for other groups, seems probable.

The present investigation was originally planned to include strains of more than one species from various sources. This was found impracticable because of the difficulty of obtaining sufficient numbers of strains. It was then decided to limit the investigation to one species. Because of its wide distribution, the certainty of identification, and availability of material for

isolation of numerous strains from different localities, *Ph. malvaceara* was chosen as the most desirable species available. All experiments were performed in the Laboratory of Plant Pathology at the University of Texas.

SOURCE OF TEST STRAINS

The strains used throughout the study were freshly isolated from young cotton plants growing in different fields in the vicinity of Austin, Texas. The organism is readily obtained from the young, water-soaked, angular spots of leaves. In order to minimize possibilities of obtaining closely related strains one strain only was isolated from each plant. The cultures were replated and fished a second time from well-separated single colonies before beginning the experiment. All of the cultures were typical of the organism described by Smith (11, pp. 314–339). Briefly, the salient features are as follows: Nitrates not reduced; starch hydrolyzed; gelatin liquefied slowly; litmus-milk alkaline with complete coagulation followed by slow peptonization; growth on potato light yellow, very copious, the water at the base becoming completely solid; growth occurs in Uschinsky's and Fermi's but not in Cohn's medium. Each strain was tested for pathogenicity by spraying water suspensions on young cotton plants growing in flower pots. The production of numerous typical water-soaked angular spots occurred after about two weeks. A total of 14 such strains was obtained.

METHODS OF CULTIVATION

All glassware used in the experiments was treated with acid-cleaning fluid and then thoroughly rinsed with tap and distilled water. The water used for preparation of media was redistilled from the stock supply. The chemicals employed were of the highest purity obtainable and further purification was not attempted.

Since the object of the investigation was to determine organic rather than inorganic requirements for growth, experiments were not performed to determine the most favorable mineral solution. The basic solution adopted was prepared according to the recent formula of Frazier and Rupp (3). This solution contains dibasic potassium phosphate 0.31 per cent, monobasic potassium phosphate 0.08 per cent, potassium chloride 0.02 per cent, and magnesium sulphate 0.02 per cent. In media containing ammonia as the only source of nitrogen, 0.25 per cent of sodium ammonium phosphate was substituted for the potassium phosphates.

The various carbon and nitrogen compounds were dissolved in these constant mineral solutions and were then distributed in 5 to 8 cc. quantities in large test tubes. The sugar-containing media were discontinuously sterilized at 100° C. Media containing urea were sterilized by filtration

and then aseptically tubed. All other media were sterilized in an autoclave at 12 to 15 pounds pressure. Reaction of the finished media varied from about pH 7.6 to pH 6.8. *Phytomonas malvaceara* grows very well in beef-extract-peptone broth at these reactions.

The sodium salts of the various organic acids were supplied in 0.2 per cent, the carbohydrates and alcohols in 0.5 per cent concentration. The nitrogen-bearing compounds were added as follows: Asparagin, glycerin, aspartic acid, glutamic acid, alanine, edestine, 0.3 per cent, urea 0.1 per cent, and tyrosine 0.1 per cent.

The inoculum was prepared by rubbing up a small bit of the growth from a 48-hour-agar-slant culture in about 3 cc. of distilled water. One loopful of the suspension was used for inoculating each tube of synthetic medium. This amount was sufficient to cause growth in control tubes of plain beef-extract-peptone broth. During the period of incubation the test-tube cultures were laid on a sloped surface to afford better aeration. Results were recorded as negative if visible growth had not occurred at the end of 30 days.

Following Braun's method of serial cultivation, transplants were made from all positive tubes as soon as the growth had apparently reached its maximum. The time varied somewhat with different strains and quite markedly for different media. Results were not recorded as positive until growth occurred in the fourth successive transplant. Agar plate cultures were prepared from the final positive tubes in order to make certain that contaminations had not been picked up in the series.

RESULTS

Results of the experiments to determine utilization of carbon compounds with ammonia or asparagin are shown in table 2. All strains behaved uniformly in all of the combinations tested.

It is seen that either ammonia or asparagin is a suitable source of nitrogen in the presence of a satisfactory source of carbon but that asparagin fails to supply both nitrogen and carbon. Growth was very copious in the case of glucose, sucrose, lactose, galactose, and levulose; less abundant but vigorous with maltose, starch, glycogen, inulin, raffinose, glycerin, and the sodium salts of acetic, citric, butyric, lactic, and succinic acids. No growth whatever occurred with arabinose, mannitol, dulcitol, salicin, formate, tartrate, or oxalate after the first transplant and there never was more than a scant, almost invisible, clouding of the medium in any of the first tubes. In all suitable media growth was as prompt and vigorous in the last set of tubes as in the original. The results would not have been changed in any way by eliminating the third and fourth serial transplants. The additional

TABLE 2.—*The growth of Phytomonas malvaceara in synthetic media containing ammonia or asparagin and various sources of carbon*

Sources of carbon	Sources of nitrogen		
	Ammonia	Asparagin	None
Asparagin		−	
Glucose	+	+	−
Levulose	+	+	−
Sucrose	+	+	−
Maltose	+	+	−
Lactose	+	+	−
Galactose	+	+	−
Arabinose	−	−	−
Raffinose	+	+	−
Xylose	+	−	−
Starch	+	−	−
Inulin	+	−	−
Glycogen	+	+	−
Glycerol	+	+	−
Mannitol	−	−	−
Dulcitol	−	−	−
Salicin	−	−	−
Sodium acetate	+	−	−
" citrate	−	+	−
" butyrate	+	+	−
" formate	−	−	−
" lactate	+	+	−
" tartrate	−	−	−
" succinate	+	+	−
" oxalate	−	−	−

nitrogen-bearing compounds were tested to determine ability to obtain both nitrogen and carbon from the same compound or nitrogen in the presence of a suitable source of carbon. The results of these tests are shown in table 3.

The fourteen strains behaved uniformly. Growth occurred with all the nitrogen-bearing compounds tested when a suitable source of carbon was present. Four only of the compounds, peptone, glutamic acid, alanine, and edestine, were capable of supplying both carbon and nitrogen.

The work of Jones, Smith, Bryan, and Honing on other species of plant-pathogenic bacteria affords some basis for comparing the utilization of salts of organic acids in the presence of ammonia. These results are given in table 4.

It is seen that both citrate and succinate are suitable for all of the species tested. Some of the compounds appear to have differential value. These

TABLE 3.—*The growth of Phytomonas malvaceara in synthetic media containing sucrose or glycerol and various sources of nitrogen*

Source of nitrogen	Source of carbon		
	Sucrose	Glycerol	No additional carbon
Peptone	+	+	+
Ammonia	+	+	–
Asparagin	+	+	–
Urea	+	+	–
Uric acid	+	+	–
Glutamic acid	+	+	+
Aspartic acid	+	+	–
Glycine	+	+	–
Alanine	+	+	+
Tyrosine	+	+	–
Leucin	+	+	–
Cystine	+	+	–
Edestine	+	+	+
None	–	–	–

results suggest the possibility of differentiating between species by differences in utilization of nitrogen and carbon compounds.

TABLE 4.—*Utilization of salts of organic acids and ammonia by four species of plant-pathogenic bacteria*

Name of organism	Carbon compounds									Author
	Acetate	Butyrate	Citrate	Formate	Lactate	Malate	Oxalate	Succinate	Tartrate	
E. carotovora	+			–						Jones
Ph. delphinii			+					+	–	Bryan
Ph. solanaceara	–	–	+		–	+		+	+	Smith
Ph. malvaceara	+	+	+	–	+		–	+	–	Lewis
Ph. solanaceara	–		+		+			+	+	Honing

SUMMARY

It has been shown that our knowledge of carbon and nitrogen utilization by species of plant-pathogenic bacteria is incomplete, due to few investi-

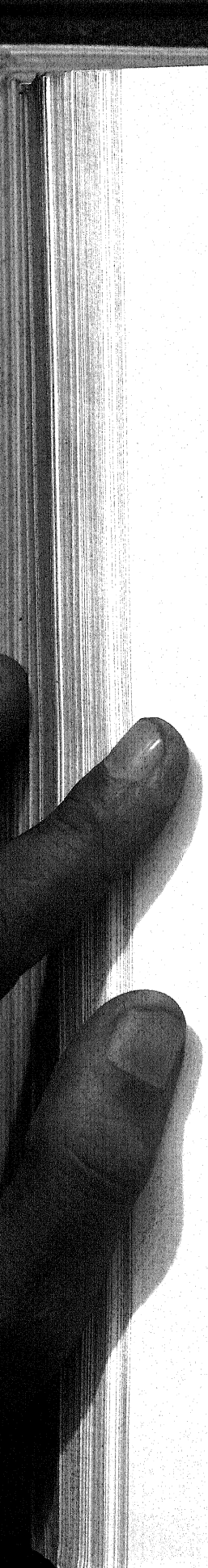
gations of the subject, the use of a too limited variety of culture media, and questionable technique.

Fourteen strains of *Phytomonas malvaceara* were found uniform in regard to carbon and nitrogen compounds that support growth. The organism assimilated ammonia and all of the various more complex nitrogenous compounds tested when supplied with various sources of carbon. Peptone, alanine, glutamic acid, and edestine support growth in the absence of additional carbon. Some of the carbon compounds tested failed to support growth in the presence of ammonia or asparagin. All results are based on ability to maintain growth through four successive transplants.

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A NEW DIPLODIA EAR ROT OF CORN

A. H. EDDINS[1]

INTRODUCTION

In August, 1928, an ear of corn was collected in the experimental plots at Gainesville, Florida, rotted and blackened by a Diplodia organism that differs from *Diplodia zeae* (Schw.) Lév. and *D. macrospora* Earle in color of mycelium and color, shape, size, and septation of spores (1). The causal fungus was also found later in the season on old dead stalks of corn. The evidence now available indicates that this new ear rot is widely distributed in the State because diseased ears have been collected in Hillsborough, Clay, Gadsen, Jackson, and Okaloosa counties, Florida.

SYMPTOMS

The mycelium of the Diplodia causing this new ear rot penetrates the cob, kernels, husks, and stalk. In contrast to the white mold formed on the kernels and husks of severely diseased ears infected by *D. zeae* or *D. macrospora* (1), the causal fungus forms a dark brown, felt-like mold on these parts (Fig. 1, A and B). The interior of the severely diseased kernel consists of a mass of blackened tissue and fungous hyphae in which pycnidia often are imbedded. All or a part of the ear may be invaded, although the butt end is most often diseased. On some ears only a few scattered kernels show the characteristic blackening. On others the diseased kernels cannot be detected until they are germinated.

Stalks usually are diseased at the lower nodes and internodes. The pith is blackened and the pycnidia are imbedded either in the outer tissue of the stalks at nodes and internodes or they occur in stromata at the nodes where sufficient moisture has been retained in the cavity formed by the stalk and sheath for the growth of the fungus on the surface (Fig. 1, C). A characteristic that distinguishes this disease on the stalk from that caused by *D. zeae* and *D. macrospora* is that the pycnidia of the causal organism which are imbedded in the stalk emerge through longitudinal cracks in the epidermis (Fig. 2), while those of the other two organisms never make elongated cracks, although, when mature, they break out through the epidermis.

The disease has not been observed on the roots of plants in the field. However, the roots of seedlings grown in sterilized sand inoculated with a pure culture of the fungus were severely rotted and blackened, and the plants were either stunted in growth or killed.

[1] Grateful acknowledgment is made to Dr. G. F. Weber for suggesting the investigation of this problem and to Dr. W. B. Tisdale for aid in preparing the manuscript.

CAUSAL ORGANISM

Morphology

Pycnidia: The pycnidia occur in clumps or series and break out through longitudinal cracks in the epidermis of old stalks or occur in stromata on the surface at nodes, on the husks, and on the surface and interior of kernels. They are subglobose to globose, black in color, and measure 170–470 μ in diameter.

Pycnospores: Young pycnospores, borne on short conidiophores, are nonseptate, colorless, and granular when young, becoming brown at maturity. Mature spores are generally uniseptate, nonseptate dark colored spores occurring rarely. The dark spores frequently have longitudinal striations on their surfaces, measure 19–31 x 11–15 μ, mostly 24–28 x 13–14 μ, and average 25.3 x 13.3 μ. The spores germinate within two to four hours in tap water at room temperature. While in the process of germination

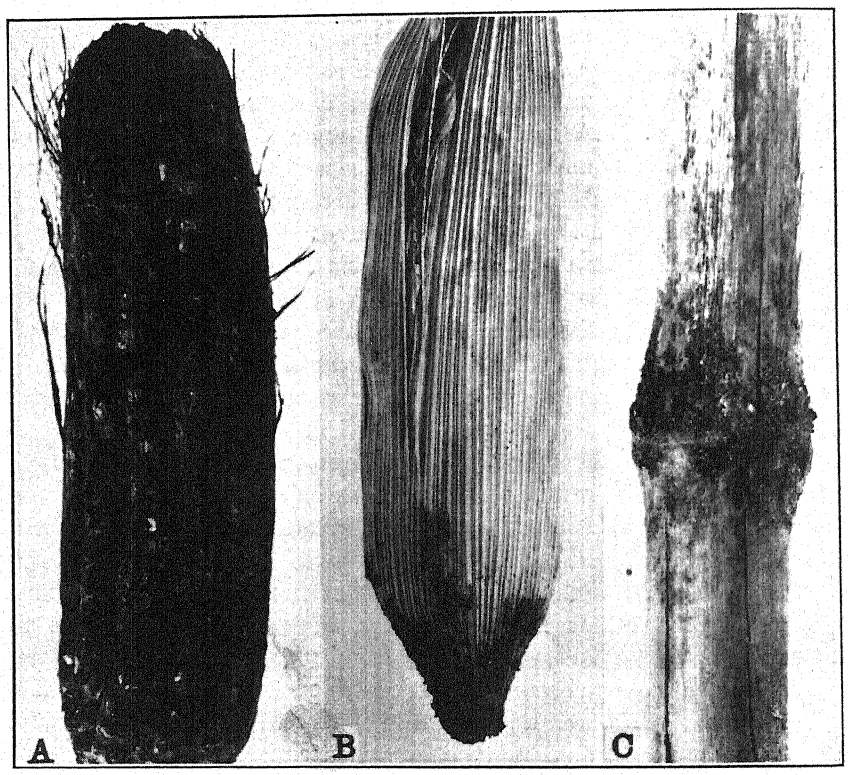


FIG. 1. A. An ear of corn severely rotted and blackened by *Diplodia frumenti*. B. Ear showing pycnidia of *D. frumenti* in stroma on the husks at the butt end. C. Pycnidia of *D. frumenti* in stroma on the surface of a stalk at the node.

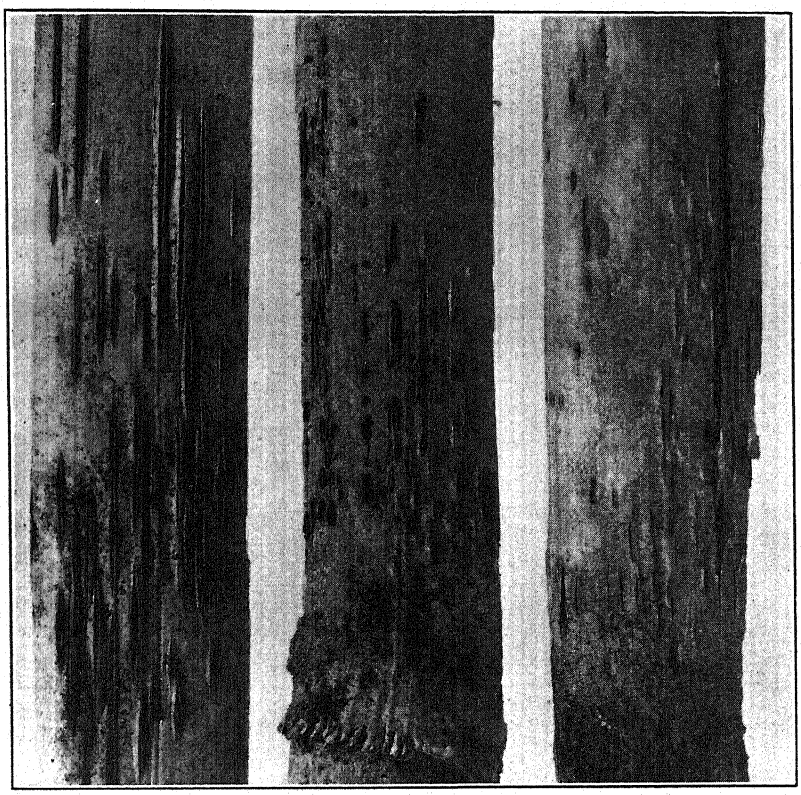


FIG. 2. Pycnidia of *Diplodia frumenti* breaking out through longitudinal cracks in the epidermis of stalks. × 2.

the spores swell at one end and the wall is slit in a ragged manner as the germ tube emerges.

Mycelium: On potato-dextrose agar, the young mycelium is hyaline and granular, septate and branched, producing a loose, flocculent, white aerial growth. As it grows older, the mycelium becomes brown and forms stromatic masses on the surface of the media. On badly diseased ears, the fungus forms brown, felty masses of mycelium on the kernels and husks.

Chlamydospores: Chlamydospores are produced in old cultures in which the nutrients have been exhausted. They are dark brown, intercalary, usually in chains.

Drawings of spores, mycelium, and chlamydospores of the fungus are shown in figure 3.

Taxonomy

In addition to *Diplodia zeae* and *D. macrospora,* one other Diplodia has been mentioned as occurring on corn. This organism was collected in 1886

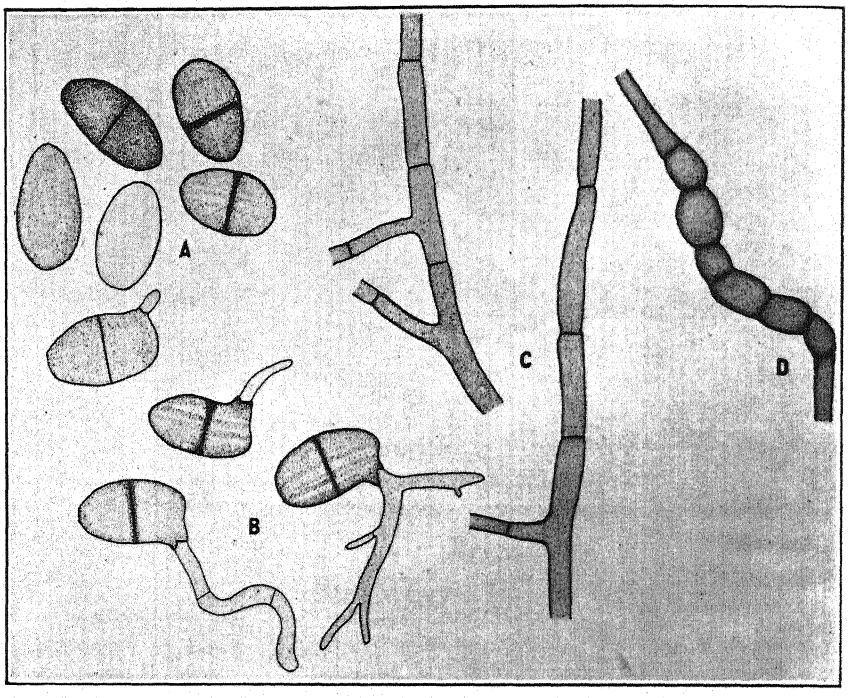


FIG. 3. Diplodia ear-rot fungus. A, various types of pycnospores; B, germinating pycnospores; C, mycelium; D, chlamydospores. × 720.

by Langlois in Louisiana on old dead stalks of corn and was described and named *D. frumenti* by Ellis and Everhart (2), whose description is as follows:

"*Diplodia frumenti,* E. and E.—On dead stalks of *Zeae mays,* June. Langlois, No. 493. Perithecia globose, sometimes with a stout, cylindrical ostiolum, subcaespitose or often seriate, bursting out through longitudinal cracks; sporules elliptical, brown, 1—septate, 15–18 x 12 μ, on stout pedicels. Very different from *Diplodia zeae,* Lév."

The Diplodia on corn in Florida agrees with the above description except that dark, nonseptate spores as well as uniseptate spores occur, and the limits in dimensions of the spores of the Florida organism exceed those of Ellis and Everhart's fungus. The spores of the Florida fungus measure 19–31 x 11–15 μ and those of *D. frumenti,* as described by Ellis and Everhart, measure 15–18 x 12 μ. The smaller size given for the latter may have been due to the possibility that only a few spores were measured and that these, if from old herbarium material, had shrunken. In obtaining the dimensions of the spores of the Florida Diplodia 200 spores from material fresh from the field were measured.

A specimen of the Diplodia on an old dead cornstalk collected from a field at Gainesville, Florida, in April, 1929, was sent to Dr. C. L. Shear, Washington, D. C., who identified it as *D. frumenti.* Pycnospores of this fungus cannot be distinguished from those of the Diplodia species which naturally occur on sweet potatoes, Citrus and cotton. They are similar in shape, septation, and color (Fig. 4) and have about the same dimensions, as is shown in table 1.

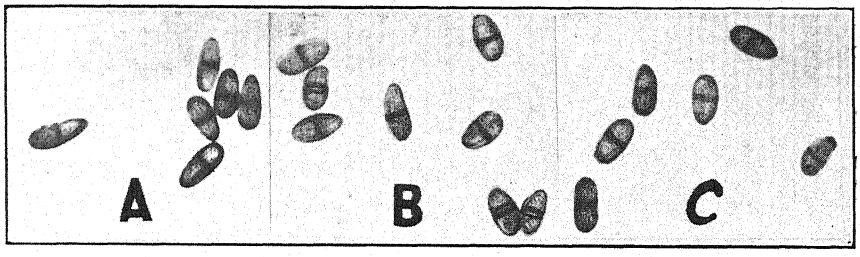


FIG. 4. Photomicrographs of pycnospores. A, *Diplodia frumenti;* B, *Physalospora rhodina;* C, *Diplodia tubericola.* ×400.

TABLE 1.—*Dimensions of 200 pycnospores each of the Diplodia species that occur naturally on corn, sweet potatoes, Citrus, and cotton when produced on ears of corn that were artificially inoculated with pure cultures of these organisms*

Source of pure cultures used for inoculation	Spore dimensions in microns	
	Limits in dimensions	Average dimensions
Corn stalks	19–31 x 11–15	25.3 x 13.3
Rotted sweet potatoes	18–31 x 9–15	24.9 x 12.5
Rotted oranges	21–30 x 10–15	27.2 x 12.6
Rotted cotton bolls	18–29 x 10–15	22.8 x 12.7

Inoculation tests have shown also that the Diplodia species on sweet potatoes, Citrus, and cotton cause a dry rot of ears of corn and the symptoms of the disease are similar to those caused by the Diplodia that naturally occurs on corn. Furthermore, the Diplodia from corn artificially inoculated into sweet potatoes, Citrus and cotton causes a rot of these hosts and the symptoms of the disease are similar to those of the diseases caused by the *Diplodia* spp. that naturally occur on these plants. Stevens (6) has shown that the common Diplodia that occurs on Citrus, *D. natalensis* Evans, and the common one on cotton, *D. gossypina* Cke., are one species, the perfect stage of which is *Physalospora rhodina* (Berk. & Curt.) Cooke. In 1911 Fawcett and Burger (3) found that Diplodia isolated from gumming peach trees caused a gumming of orange trees and Diplodia isolated

from orange trees caused a gumming of peach trees. Other workers (4, 5, 7) have also shown that *D. natalensis* and *D. gossypina* cause a rot of sweet potatoes similar to the rot caused by *D. tubericola* Ell. and Ev., and *D. tubericola* caused a stem-end rot of watermelons. It is possible that only one fungus is being dealt with here, as the organisms pass readily from one host to another. However, it has not been demonstrated that the Diplodia on corn and that on sweet potatoes have *P. rhodina* as their perfect stage. In view of this fact the wisest course at present seems to be to consider *D. frumenti* on corn as a species distinct from *D. tubericola* and *P. rhodina*.

Germination of the spores

Spores of *D. frumenti* were germinated in tap water in drop cultures exposed to the following temperatures Centigrade: 5°, 8°, 15°, 20°, 23°, 25°, 28°, 32°, 35°, and 39°. The daily fluctuations in these temperatures were not more than plus or minus one degree. The percentage germination at each temperature at the end of four hours is given in table 2. When the same cultures were held at the various temperatures for six days, the spores germinated at temperatures as low as 8° C.

TABLE 2.—*Percentage germination of spores of Diplodia frumenti exposed to different temperatures for four hours*

Temperatures in degrees Centigrade	5	8	15	20	23	25	28	32	35	39
Percentage germination	0	0	0	0	11	28	40	32	27	11

Growth on culture media

Diplodia frumenti was grown on sterilized apple, sweet-potato, and orange plugs and sterilized wheat and corn kernels. Pycnidia were formed by the organism on all these media except the orange plugs. When grown on corn meal, carrot, oatmeal, glycerine, lactose, potato-dextrose, and malt-extract agars at room temperature, the fungus formed dark brown stromatic masses of mycelium on the surface of these media and produced pycnidia on all except the lactose and malt-extract agars.

Relation of temperature to mycelial growth

A series of triplicate plates of potato-dextrose agar were inoculated in the center with uniform squares of a young agar culture of the organism and incubated for 48 hours at temperatures ranging from 5° to 39° C. Maximum growth, as indicated by the diameters of the colonies in table 3, occurred between 25° and 32° C. The fungus grew slightly at 39° C., and the lower limit at which growth occurred was 15° C.

TABLE 3.—*Relation of temperature to the mycelial growth of Diplodia frumenti on 2 per cent potato-dextrose agar*

Time of reading	Diameter of colonies in centimeters at temperatures expressed in degrees Centigrade									
	5	8	15	20	23	25	28	32	35	39
24 hours	0	0	0.5	1.7	2.5	3.7	4.2	5.0	1.1	trace
48 hours	0	0	1.8	4.3	6.5	8.3	8.7	9.0	1.7	0.3

Relation of hydrogen-ion concentration to growth

Diplodia frumenti was grown on a 2 per cent potato-dextrose agar adjusted to different hydrogen-ion concentrations. Fifteen cubic centimeters of the agar were placed in each of 120 test tubes, and, after sterilization, the adjustment of the pH concentration of each tube was made by adding the required number of drops of 2N, N/1, N/3, HCl, and N/1 NaOH. The amount of acid and alkali required to adjust the media to the different concentrations was determined with the Youden Hydrogen-Ion Concentration Apparatus. Petri plates were prepared in triplicate for each reaction, inoculated in the center with uniform bits of fungous mycelium, and exposed at a constant temperature of 30° C. for 48 hours. According to the diameters of the colonies given in table 4, the fungus made about the same rate of growth at pH concentrations ranging between 4.9 and 7.6. The organism grew well at pH 8.2, but growth was inhibited at pH 1.9, the lower limit.

TABLE 4.—*Relation of hydrogen-ion concentration of 2 per cent potato-dextrose agar to the mycelial growth of Diplodia frumenti at 30 C.*

pH	Average diameter of colonies in centimeters after	
	24 hours	48 hours
1.9	0	0
2.9	0	trace
3.5	1.8	4.5
4.6	2.3	5.1
4.9	2.8	5.6
5.2	3.0	5.6
6.0	2.8	5.7
7.1	3.2	5.8
7.6	3.2	5.8
8.2	1.5	4.5

Pathogenicity on corn

Ears of corn in the soft-dough stage were inoculated with *D. frumenti* by the following methods: (1) A kernel of corn on which a pure culture of the fungus was growing was inserted into a wound made in the husks and pressed down in contact with the kernels; (2) a water suspension of spores was sprayed on the exposed tips of ears; and (3) a water suspension of spores was hypodermically injected into the husks of ears near the butt end. As noted in table 5, ears were infected when inoculated by each method. The diseased ears had some or all of their kernels severely rotted and blackened by the organism which was recovered in pure culture when infected kernels were plated on agar. The data show that the fungus may gain entrance to the ears through exposed tips and wounds. Ears in the field often are diseased only at the butt ends. This indicates that the spores lodge in the cavities formed by the husks and ear sheath at the butt, where they germinate, and the fungus grows into the ear.

TABLE 5.—*Results obtained by inoculating ears of corn in the soft-dough stage with mycelial cultures and spore suspensions of Diplodia frumenti*

Method of inoculation	Number of ears inoculated	Number of ears infected	Number of ears on which pycnidia appeared
Mycelial cultures inserted into wounds in husks	66	42	7
Spore suspension sprayed on exposed tips	81	11	6
Spore suspension injected into husks near butt end	79	18	9

Healthy corn seedlings with roots from one to two inches long were set out in sterilized white lake sand and in sand sterilized and inoculated with a pure culture of *D. frumenti*. At the end of two weeks the roots of seedlings grown in sand inoculated with the organism were severely rotted and blackened and the tops of the plants were either wilted or dead. Seedlings in the noninoculated sand were healthy. In this experiment the amount of inoculum was greatly in excess of what normally occurs in the field. Consequently, the test indicates only the parasitic capabilities of the fungus and not its pathogenicity under normal field conditions.

Pathogenicity on other plants

The pathogenicity of *D. frumenti* was tested on oranges, grapefruits, sweet potatoes, cotton bolls, and watermelons. Inoculations were made

under sterile conditions by inserting mycelium from pure culture into wounds made in each fruit with a sterilized scalpel. As shown in table 6, all these fruits were rotted by the organism, thus demonstrating that *D. frumenti* is not confined to corn but can cause a typical Diplodia rot of oranges, grapefruits, sweet potatoes, cotton bolls, and watermelons.

TABLE 6.—*Results of inoculating oranges, grapefruits, sweet potatoes, cotton bolls, and watermelons with mycelium of Diplodia frumenti*

Fruits inoculated	Number inoculated	Number rotted	Number noninoculated (checks)	Number rotted (checks)
Oranges	8	8	12	0
Grapefruits	3	3	2	0
Sweet potatoes	14	10	15	0
Cotton bolls	22	22	24	3[a]
Watermelons	3	3	2	0

[a] The cotton was inoculated in the field and the 3 rotted bolls of the noninoculated checks were naturally infected.

In another test, mycelium from a pure culture of *D. frumenti* was inserted into wounds made in the bark of young orange twigs. The wounds were wrapped with absorbent cotton which was moistened each day. At the end of three weeks gummosis and necrosis of the twigs had occurred in the region of the wounds and at ten weeks pycnidia containing mature spores had been formed. The noninoculated wounds that served as checks healed and no symptoms of Diplodia infection were evident.

Overwintering

Diplodia frumenti survives the winter in Florida on old plant débris in the field, for ear infection has been obtained from spores collected from old stalks left in the field until June. It also lives over as dormant mycelium in the seed.

SUMMARY

A hitherto unknown Diplodia ear-rot of corn has been found in Florida. The stalks also are attacked. The symptoms of the disease and morphology of the fungus are described.

The causal organism agrees closely with *Diplodia frumenti* E. and E. and is referred to that species. Cross inoculations showed that *D. tubericola, D. natalensis*, and *D. gossypina* cause a rot of ears of corn similar to that caused by *D. frumenti*, and all forms cause a similar rot of oranges, grapefruit, sweet potatoes, cotton bolls, and watermelons. The four

organisms resemble each other in their imperfect stages. *D. natalensis* and *D. gossypina* are synonymous and have *Physalospora rhodina* as the perfect stage. *D. frumenti* and *D. tubericola* are tentatively considered two distinct species because their perfect stages are unknown.

Spores of *D. frumenti* germinate best at temperatures ranging between 25° and 32° C. The fungus grows well on standard culture media. The temperatures at which maximum growth occurs on potato-dextrose agar range between 25° and 32° C., and the pH reaction most favorable for growth lies between pH 4.9 and 8.2.

Diplodia frumenti enters the ears through the exposed tips and wounds and by growing into the ears at the butt ends. The organism hibernates in old cornstalks in the field and as dormant mycelium in the seed.

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THE IDENTITY OF THE POTATO BLACKLEG PATHOGENE[1]

J. G. LEACH

The question of the correct name to be applied to the blackleg pathogene has been the subject of much discussion and the cause of considerable confusion. Frank (3) in 1899 was perhaps the first to publish a recognizable description of the disease and to attribute it to a definite bacterial pathogene. He described briefly a Micrococcus, which he called *M. phytophthorus*. Since no later workers have found a Micrococcus to be pathogenic to potatoes and since abundant evidence has been presented to show that blackleg is due to a Bacillus, it is to be concluded that Frank's organism was not the true pathogene.

In 1902, van Hall (4) described a Bacillus as the pathogene and named it *Bacillus astrosepticus*. Shortly before this, Appel (1) reported the pathogene as *Bacillus phytophthorus* but did not publish a complete description until 1903 (2). Harrison (6), in 1907, published a detailed description of blackleg as it occurred in Canada. He described the pathogene as a Bacillus slightly different from those already reported and named it *B. solanisaprus*. In 1911, Pethybridge and Murphy (16) attributed the disease in Ireland to *B. melanogenes*, which they found to differ slightly from the previously described organisms. Morse (14), in 1917, published the results of comparative studies of *B. atrosepticus* van Hall, *B. solanisaprus* Harrison, *B. melanogenes* Pethybridge and Murphy, and three cultures isolated from diseased potatoes in Maine. Although some slight differences were found, such as "slight variations in size, shown more particularly by *B. solanisaprus* and IIIA, and the production of a slight viscidity on different kinds of media shown by *B. solanisaprus* and *B. melanogenes*," he concluded that they "should be classed as one species or at the most, strains of the same species." On the grounds of priority the name *B. astrosepticus* van Hall was held to be valid. Unfortunately, an authentic virulent culture of *B. phytophthorus* Appel was not included in the comparison.

Jennison (7), however, has repeated and extended this phase of Morse's work, comparing twelve strains of the pathogene, including a virulent culture of Appel's original strain of *B. phytophthorus*, as well as cultures of the other three so-called "species." Jennison agreed with Morse that the slight differences were not sufficient for species characterization. He also agreed with Morse that the pathogene should bear the name *B. astro-*

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septicus van Hall. Smith (19), however, preferred to use the name *B. phytophthorus* Appel.

Paine and Chaudhuri (15), in 1923, using a culture of *B. astrosepticus* isolated and identified by Paine in 1917 and one of *B. solanisaprus* obtained in the same year from the American Museum of Natural History, found the two to differ in several important respects, such as resistance to heat, type of decay on potato tubers and stems, and gelatine liquefaction.

St. John-Brooks, Nain, and Rhodes (17), in 1925, compared several strains of *B. carotovorus,* two strains of *B. phytophthorus,* and one of *B. solanisaprus.* All strains except one of *B. carotovorus* produced identical reactions in sugar media and behaved alike on other media but were found to differ serologically.

In the course of a study of the relation of the seed-corn maggot and potato blackleg, the writer had occasion to isolate a large number of cultures of bacteria from the different stages of the insect, from the soil, and from potatoes affected with blackleg. Many of the cultures proved pathogenic to potato tubers and stems. Since *B. carotovorus* Jones is known to be pathogenic to potatoes and since the seed-corn maggot is known to breed in plants which are recognized hosts for *B. carotovorus,* it was expected that some of the cultures would prove to be this organism. Therefore, an effort was made to distinguish *B. carotovorus* from the blackleg pathogene, but it was found impossible to do so. Although there were slight variations in minor cultural characteristics among the cultures isolated, they all had the same primary characteristics as indicated by the index number[2] 5010–32120–0111. For closer comparison, a culture of *B. carotovorus* Jones was obtained from Professor A. B. Massey. This was said to be a subculture of Jones's original type culture designated as 3a (12). This culture was found to be identical with an authentic culture of the blackleg pathogene (*B. phytophthorus* Appel I) obtained by the writer from E. F. Smith, in so far as primary characteristics were concerned, although there were a few differences in minor cultural characters. The most pronounced difference was the production of less black pigment by *B. carotovorus* Jones 3a on potato tubers and stems. When this character was used in an attempt to identify the unknown cultures so many intermediate forms were found that it was impossible to do so. Massey (12) was able to distinguish *B. phytophthorus* and *B. carotovorus* on the basis of the production of acid by the latter on 5 per cent alcohol agar. This test was used by the writer, also, but numerous other pathogenic cultures isolated from rotting carrots, cabbage, celery, onions, and iris, otherwise answering the description of *B. carotovorus,* did not show this property. Since there appeared

[2] The descriptive chart of the Society of American Bacteriologists.

to be strains of *B. carotovorus* that did not possess this property it was felt that the test could not be relied on for identifying the species.

Kotila and Coons (10) showed that *B. astrosepticus* produced a toxic substance that would diffuse through a colloidal membrane and cause potato leaves to wilt. They also showed that 5 cc. of a broth culture of *B. astrosepticus* added to a nutrient solution containing the uninjured roots of a potato plant would not infect the plant but would produce a substance that was toxic to it. They conclude that, "*B. astrosepticus* seems to possess the power of producing a toxic substance which affects potato cells so as to allow blackening, probably oxidation, to ensue, while *B. carotovorus* which softens potato tissues under some conditions without blackening, does not possess this power."

These authors, however, did not report any experiments to show whether *B. carotovorus* would likewise produce a toxic substance. The writer, therefore, made experiments both with *B. carotovorus* and *B. astrosepticus*. It was found that both cultures produced a toxic substance that wilted cut leaves and caused the yellowing and ultimate death of plants grown in water cultures to which 5 cc. of broth cultures had been added. This property therefore could not be used in identifying the cultures.

The similarity between the blackleg pathogene and *B. carotovorus* Jones, the cause of a soft rot of many succulent plants has been recognized by many workers. After comparing several cultures of bacteria causing soft rots, Smith (19), stated that *B. phytophthorus* was "not sufficiently distinguished from *Bacillus carotovorus* Jones, which name is earlier."

Harding and Morse (5), working in collaboration with Jones, made a detailed physiological study of forty-three strains of bacteria causing soft rot of plants. They found that the strains could be separated into six groups on the basis of their action on dextrose, lactose, and saccharose. They did not consider these differences sufficient to justify the recognition of six different species but regarded the cultures as somewhat variant strains of a single species. Jones (9) also made a study of the enzyme activity of the same strains studied by Harding and Morse. He found considerable variation in this respect and presented the results as "contributing further evidence to the general likeness of these strains and partly as emphasizing the minor variations which we believe must always be expected to occur with different bacterial strains, even of the same so-called species." These differences, however, are equally great, as the differences on which the various "species" of the blackleg pathogene have been distinguished from each other and from *B. carotovorus*. To facilitate comparison the index numbers of the principal soft-rotting bacteria as determined by various workers have been assembled in table 1.

From this summary it will be noted that the chief point of difference between *B. carotovorus* 3a and the various "species" of the blackleg pathogene is in their diastatic properties. Jones (9), Harding and Morse (5), Smith (19), and Mehta (13) report *B. carotovorus* Jones as possessing diastatic properties usually designated as weak. Harrison (6), Morse (14), Mehta (13), and Pethybridge and Murphy (16) attribute diastatic action to the blackleg pathogene also, while Jennison (7), Shapovalov and Edson (18), and van Hall (4) found no action on starch by the latter organism. The present writer when comparing the two organisms side by side was unable to obtain a positive diastatic reaction with either. A study of the literature shows that these differences can, in all probability, be attributed to differences in method. Those workers reporting positive diastatic action have generally used the old method in which the organism is cultured on potato plugs and later treated with an alcoholic solution of iodine, decolorization of the iodine solution being considered evidence of diastatic action. Later workers generally have used the starch-agar method recommended by the Society of American Bacteriologists. By this method both cultures react negatively.

A careful comparison of the data published by various workers dealing with the various soft-rotting bacteria reveals the following facts:

1. The differences between the various "blackleg pathogenes" as described by different workers are as great as or greater than those which are supposed to distinguish them from *B. carotovorus.*
2. The differences found by various workers using, presumably, the same organisms are as great as or greater than those used for distinguishing the blackleg pathogenes from *B. carotovorus.*
3. The differences between the six groups of soft-rotting bacteria described by Harding and Morse, of which *B. carotovorus* is the type, are greater than those which are used for distinguishing the blackleg pathogenes from them.
4. The revised description of the blackleg pathogene as given by Jennison (7) agrees in all essential details with the recognized characteristics of *B. carotovorus.*

Many workers have observed the morphologic and physiologic similarity of these organisms, but they usually have considered slight physiologic differences sufficient for differentiating two separate species. The recognition of these slight physiologic differences by different specific names has apparently carried with it an implication of parasitic differences. Jones (8), in his first paper on *B. carotovorus,* reported unsuccessful attempts to infect potato tubers but, in a footnote, states that later inoculations were successful. Smith (19) later showed that this organism was readily pathogenic to potato tubers and stems. However, it was not until 1926

TABLE 1.—*Summary of the primary characteristics (represented by index numbers) of various soft-rotting bacteria, as described by various workers*

Organism	Index number	Authority	Organism	Index number	Authority
B. carotovorus, Jones 3a	5011[a]–32120–1111	Jones	*B. phytophthorus* Appel	5U1U–32120–UUU1	Appel
do	5010–32120–1111	Harding and Morse	do	5010–32120–U111	Smith
do	5010–32120–1111	Smith	do	501U–3U120–U111	Stapp
			do	5010–32120–1111	Mehta
do	5010–32120–1111	Mehta	do	5010–32120–0111	Shapovalov and Edson
do	5010–32120–0111	Leach	do	5010–32120–0111	Jennison
do, Vermont XLVIII	5010–32120–1112	Harding and Morse	do	5010–32120–0111	Leach
do, Vermont C	5010–32120–1121	do	*B. atrosepticus* v. Hall	5010–32120–0121	van Hall
do, Vermont LIV	5010–32120–1211	do	do	5010–32125–1111	Morse
do, Potter's Bacillus	5010–32120–1212	do	do	5010–32120–1121	Harrison
B. solanisaprus, Harrison	5010–32120–1212	Harrison	do	5010–32120–0111	Jennison
do	5010–32125–1111	Morse	*B. melanogenes* P and M	5010–32110–1111	Pethybridge and Murphy
do	5010–32120–0111	Jennison	do	5010–32125–1111	Morse
do	5010–32120–0111	Leach	do	5010–32120–0111	Jennison

[a] Figures in bold face type indicate points of difference when compared with the findings of the writer and the revised description of the blackleg pathogene by Jennison.

(11) that a natural infection of potato tubers due to *B. carotovorus* was reported. In so far as the present writer has been able to learn, no one has yet recorded in literature a natural primary infection of potato stems due to *B. carotovorus*. Is this due to the inability of *B. carotovorus* to infect the potato plant under natural conditions, or have we been deceived by the names applied to these organisms and assumed that all soft rot of carrots is due to *B. carotovorus* and that all soft rot of potato stems is due to *B. phytophthorus*?

If it is true that *B. carotovorus* is pathogenic to potato tubers and stems and that the blackleg pathogene is pathogenic to carrots and other vegetables, it does not seem reasonable that they should be so closely specialized in their attack on these plants in nature. In studying this question several strains of virulent soft-rotting bacteria were isolated from carrots, onions, cabbage, celery, iris, and potato tubers and stems. Cross inoculations were made and all of the cultures, without exception, were found strongly parasitic on all of the hosts. Some slight differences were observed in minor cultural characters, but all the cultures were identical in so far as the major physiological tests were concerned. They all had the index number 5010–32120–0111. Some slight differences in color of the decayed tissues were observed, but this character was very variable, being influenced by the variety of host plant used, the age of tissue, the humidity of the incubation chamber, etc. It was quite evident, however, that certain of the cultures differed in amount of pigment produced under the same conditions.

One of the most consistent differences between *B. carotovorus* and the blackleg pathogene as reported by various writers is the so-called "inky black" discoloration of tissues affected with blackleg, which is absent in tissues affected with *B. carotovorus*. The culture used by the writer, designated as *B. carotovorus* Jones 3a, was definitely characterized by the production of less pigment than most of the cultures studied. On the other hand, several cultures obtained from carrots, onions, and cabbage produced more pigment than certain cultures isolated from typical blackleg plants. It should be stated here also that the inky black discoloration so frequently mentioned in descriptions of blackleg is not always characteristic of the disease as it occurs in Minnesota. Potato tissue killed by almost any organism will turn black when thoroughly dried and oxidized, but tissues while in the process of decay by the blackleg pathogene are more frequently dull brown than inky black. The black color usually appears after the tissues have become relatively dry.

Lacey (11) compared *B. carotovorus* with several other soft-rotting organisms and concluded that "cultural, pathological, and serological tests all show that a close relationship exists between the three species, *B.*

carotovorus, B. solanisaprus, and *B. phytophthorus,* but there are nevertheless sufficiently marked and constant differences to warrant their continued separation into different species." Stapp (20), on the other hand, has compared many strains of the blackleg pathogene with *B. carotovorus* and other similar organisms. He classified them into five groups on the basis of serological and physiological differences but proposed to include all five groups in one common and uniform "*Bacillus phytophthorus*-group." Inasmuch as our concept of what constitutes a species among the bacteria is extremely vague, it appears to be only a matter of opinion as to whether a distinct specific name should be given to each of these soft-rotting organisms. The writer is inclined to favor the idea proposed by Stapp of including all in one species, but on the basis of priority it appears that *B. carotovorus* would be the correct name because *B. carotovorus* was described two years before *B. phytophthorus.*

Stapp (20) recognizes that the name *B. carotovorus* is earlier but feels that it should not be used because:

". . . 1. Jones seinem Bakterium einen von der Kulturpflanze (der Karotte), von der er den Erreger isoliert hatte, abgeleiteten Namen gegeben hat und garnicht wusste, dass dieser Mikroorganismus auch eine wirtschaftlich bedeutend wichtigere Krankheit hervorzurufen imstande ist und (dass) 2. der allergrösste Prozentsatz der zahlreichen von mir vergleichend geprüften Bakterienstämme serologisch völlig mit dem Originalstamm von *Bac. phytophthorus* Appel übereinstimmt. . . ."

The writer has no desire to quibble nor to add to the great amount of confusion already existing in regard to the name of the blackleg pathogene, but it does not appear to him that the above reasons are sufficient for disregarding the priority of the name *B. carotovorus* Jones.

At any rate, it must be recognized that potato blackleg is a bacterial soft rot and may be caused by several closely related strains of bacteria and that these same bacteria may cause a soft rot of many other plants. If we separate the "soft rot" group of bacteria into seperate species on the basis of minor physiological differences we must recognize that potato blackleg can be caused by *B. carotovorus* as well as by *B. phytophthorus.*[3] In other words, potato blackleg is considered to be nothing more than "soft rot" of the potato. The distinctive characters of the disease are due primarily to the nature of the host plant rather than to the causative agent. The potato being propagated by tubers is more subject to infection than

[3] *Bacillus aroideae* Townsend has not been considered in this discussion. It has been isolated only a few times in the course of this work. There appears to be some justification in considering this organism as a distinct species on the basis of its reaction on sugar media and of other cultural characteristics. It is, however, pathogenic to potatoes.

most plants propagated by seed. The transmission of the organism through infected seed pieces and the tendency of decayed potato tissue to form a black pigment upon oxidation have been factors contributing to the distinctive character of the disease when affecting the potato.

SUMMARY

The similarity of the bacteria causing potato blackleg to *B. carotovorus* causing soft rot of carrots and other vegetables led to a comparative study of numerous strains of pathogenic bacteria isolated from various host plants. A comparison of the morphologic, physiologic, and parasitic characteristics of these cultures showed that there is little justification for recognizing different species within the group. All of the bacteria studied were equally pathogenic to potatoes and a large group of succulent vegetables and were identical in so far as primary characters are concerned. There appears, however, to be a fairly large number of strains varying in minor characteristics, but there is no evidence of parasitic differences between those isolated from potatoes and those isolated from other vegetables. The conclusion is reached that blackleg is nothing more than soft rot of potato and that the bacteria previously designated as *B. phytophthorus* Appel, *B. astrosepticus* van Hall, *B. solanisaprus* Harrison, and *B. melanogenes* Peth. and Murph. are merely strains of the earlier-described species, *B. carotovorus* Jones.

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PHYTOPATHOLOGICAL NOTES

Ineffective nature of iodine dust as a fungicide against Tilletia caries.—In 1928 it was suggested to the writer that iodine dust, mixed with an infusorial earth, might be useful as a fungicide against bunt of wheat. In order to test this chemical the following plots were laid down: Little Joss wheat was contaminated with bunt spores from the variety Victor at a rate, approximately, of one part of bunt balls to twenty-five parts of wheat. This sample of wheat was divided into ten portions. Of these two were treated with copper carbonate dust, two with 1½ per cent of iodine dust, two with 3 per cent of iodine dust, and two with 5 per cent iodine dust, and the remaining two were not treated. The treated samples were dusted in each case at the rate of 2 ozs. of dust to the bushel of wheat. Five plots were sown in the winter, 28th of November, 1928, and five in the spring, 15th of March, 1929. Each of the winter-sown plots consisted of twelve rows, a row being ten feet; but each spring-sown plot consisted of three ten-foot rows. At harvest a total head count was made and the percentage of bunted heads determined. The results of these experiments are given in table 1. From these results it appears that iodine dust is of no use as a fungicide for *Tilletia caries*. It is stated, however, by Sayre and Thomas[1, 2] that excellent results were obtained against oat smuts by the use of iodine vapor dust, made by mixing finely ground solid iodine with infusorial earth, the dust containing 5 per cent by weight of iodine. On the other hand, Horsfall[3] states that under the conditions of his Ithaca experiments, iodine dust was ineffective. Since these American results are antagonistic, this writer thought it would be of interest to communicate the results of his experiments on the use of iodine dust against bunt in wheat.—W. A. R. DILLON WESTON, M.A., School of Agriculture, Cambridge, England.

[1] Sayre, J. D., and R. C. Thomas. New dust treatment for oats smuts. Science n. s. **66**: 398. 1927.

[2] ———. New dust treatments for oat smuts. Phytopathology **18**: 189. 1928.

[3] Horsfall, J. G. Dusting seed for oat smuts. Phytopathology **19**: 173–175. 1929.

TABLE 1

Winter-sown Plot No.	and variety	Treatment	Percentage of bunted heads
1586	Little Joss contaminated	2 ozs. of	2
1587	with Victor bunt	copper carbonate	0
1588		to the bushel of	4
1589		wheat	Less than 1
1590			1
1591			0
1592			0
1593			0
1594			0
1595			0
1596			0
1597			3
1598	Little Joss contaminated	2 ozs. of iodine dust,	9
1599	with Victor bunt	3 per cent, to the	11
1600		bushel of wheat	12
1601			13
1602			4
1603			13
1604			9
1605			4
1606			3
1607			12
1608			12
1609			17
1610	Little Joss contaminated	2 ozs. of iodine dust,	15
1611	with Victor bunt	1½ per cent, to the	11
1612		bushel of wheat	8
1613			16
1614			14
1615			7
1616			19
1617			5
1618			14
1619			7
1620			9
1621			15
1622	Little Joss contaminated	2 ozs. of iodine dust,	17
1623	with Victor bunt	5 per cent, to the	6
1624		bushel of wheat	3
1625			8
1626			15
1627			17

TABLE 1—(*Continued*)

Winter-sown Plot No.	and variety	Treatment	Percentage of bunted heads
1628			8
1629			17
1630			5
1631			14
1632			4
1633			15
1634	Little Joss contaminated	Nontreated	2
1635	with Victor bunt		15
1636			8
1637			8
1638			11
1639			13
1640			12
1641			14
1642			16
1643			20
1644			8
1645			16
Spring-sown			
2175	Little Joss contaminated	2 ozs. iodine dust,	0
2176	with Victor bunt	1½ per cent, to the	0
2177		bushel of wheat	2
2178	Little Joss contaminated	2 ozs. iodine dust,	0
2179	with Victor bunt	3 per cent, to the	0
2180		bushel of wheat	0
2181	Little Joss contaminated	2 ozs. copper carbonate	0
2182	with Victor bunt	dust to the bushel	Less than 1
2183		of wheat	0
2184	Little Joss contaminated	Nontreated	0
2185	with Victor bunt		Less than 1
2186			2
2187	Little Joss contaminated	2 ozs. iodine dust,	11
2188	with Victor bunt	5 per cent, to the	6
2189		bushel of wheat	3

A Bacterial Disease of Tung-Oil Tree.—In October, 1929, the writer observed for the first time a prominent spotting of the leaves of the tung-oil, or wood-oil, tree (*Aleurites fordi* Hemsl.)in southern Georgia. The spot was readily recognized as bacterial in character because of the immense number of bacteria in young and moderately old lesions and of the almost total absence of other organisms. This determination was confirmed by Lucia McCulloch, who examined material submitted to the Office of Mycology and Disease Survey, United States Department Agriculture. Necrotic lesions were present, also, on the trunk and petioles of young trees heavily affected with the leaf spot and are suspected to have the same origin.

The occurrence of this disease seems to be of considerable interest, inasmuch as there appear to be no bacterial diseases described for any of the five species listed for Aleurites (*A. fordi* Hemsl., *A. montana* (Lour) Wils., *A. cordata* R. Br., *A. moluccana* Willd. [syn., *A. triloba*], and *A. trisperma* Blanco [syn., *A. saponaria*]) in their native habitats or elsewhere; and no bacterial leaf spots for any of the related plants, *i.e.*, Euphorbiaceae, in this country. For conditions in Florida,[1] the belief has been expressed that *A. fordi,* on being introduced into the United States, brought none of its native insect pests or diseases with it, and, at that time, the plant in Florida showed no disease except the common root-knot disease (*Caconema radicicola*). A survey of tung-oil plantings in southwestern Georgia revealed the presence of the leaf spot, together with bark and petiole lesions, in each of the three nurseries inspected, and the leaf spot in all five orchard plantings visited, ranging in age from two to eight years, as well as in most of the scattered ornamental trees of various ages and locations. The oldest trees on which leaf spot was observed were isolated plants reported to be about thirty years old. The disease was prominent, however, and notably injurious in only one planting, *i.e.*, a thickly planted nursery of one season's growth containing comparatively rapidly growing, succulent trees. It seems, therefore, that the disease may not be necessarily very recent in its occurrence in the State or limited in its distribution.

In regard to the economic importance of the disease, it might be stated that in case the tung-oil industry in the Gulf States continues to increase in extent as it has during the past few years, it may prove of some importance.

The manner in which the disease is spread is not known. The fact, however, that it occurs in Georgia in nearly all plantings examined, suggests its probable occurrence on other common host plants or that the causal organism may be readily seed-disseminated.

The leaf spots are characteristic of the angular type of bacterial lesions on such organs, are decidedly delimited by veins (Fig. 1, A), brown to black on the upper side and yellowish brown on the lower surface, and are sur-

[1] Newell, W. The tung-oil tree in Florida. Fla. Agr. Exp. Sta. Bul. 171. 1924.

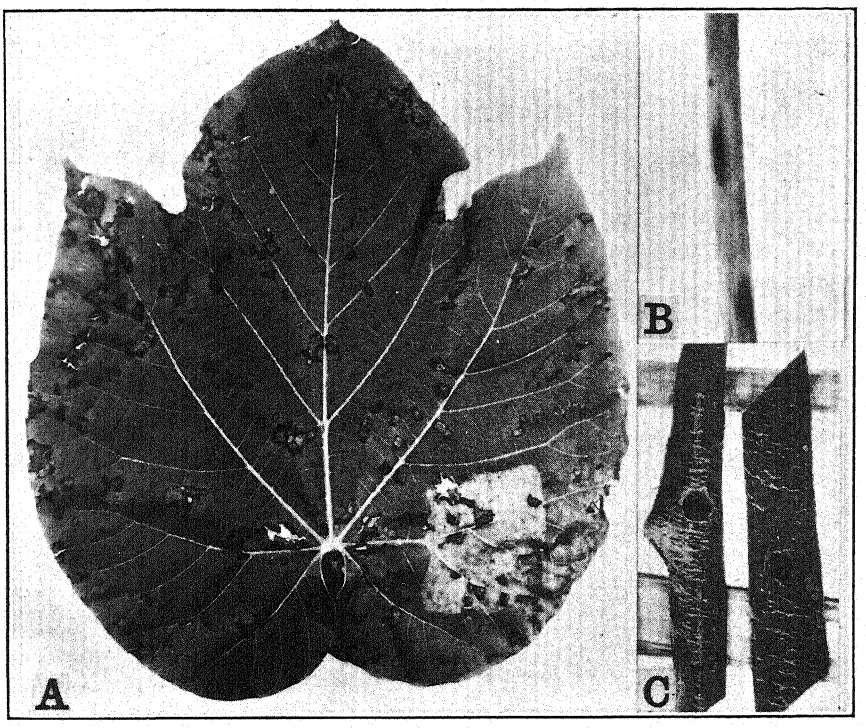


FIG. 1. A. Upper surface of tung-oil leaf showing characteristic spotting by the bacterial disease. Center of older lesions, reddish brown to yellowish brown in color. B. Lesions on a leaf petiole. C. Trunk bark of trees affected with the bacterial leaf spot. A, about $\frac{1}{3}$ natural size. B and C, about natural size.

rounded by a slightly water-soaked, faintly yellowish halo of indefinite width. The lesions may coalesce to form large areas of dead, easily broken tissue, thus imparting a torn and ragged appearance to the leaf. The accompanying lesions on the petioles (Fig. 1, B) are long, and brown and extend into the cortex; those on the bark (Fig. 1, C) round to oblong, brown to black in color, extending into the wood.

In the heavily infected nursery planting referred to above, practically every tree in the three to four acre plot showed leaf infection, and many plants, notably those in certain scattered spots that appeared to represent points of primary infection, showed all of their leaves severely spotted and many badly torn. There was indication that secondary spread had been brought about by wind-blown rain and by workmen and teams during cultivation. Observations in general suggested that only relatively young tissues of well-nourished, rapidly growing young trees are likely to undergo severe injury during such a season. In no instance did trees four years old

or older show marked injury, regardless of health or rapidity of growth.—O. C. BOYD, *Formerly Plant Pathologist, Georgia State Board of Entomology.*

Some microscopic characters of the rot caused by Ganoderma Curtisii. *Ganoderma Curtisii* (Berk.) Murrill is very common on various species of hardwood trees along the southern Atlantic coast and extends inland as far as western Texas. It is easily identified by certain well-marked gross characters that differentiate it from all other species of Ganoderma. It frequently occurs on various species of oak, usually on the stumps and on old wounds at the base of living trees. It is one of the principal fungi that rot the roots of dead oak trees, thereby loosening them so that the stumps can be easily pulled from the soil.

Macroscopic characters of the rot. This fungus usually produces a soft indeterminate white rot with gross characters but little different from other similar rots of dead timber.

Microscopic characters. The characters herein given were taken from an early stage of the rot found in live oak, *Quercus virginiana* Mill.

When the vessels are treated with phloroglucin they stain a deep pink and show no indication of any corrosion of their bordered pits; no cellulose reaction obtains. The middle lamellae of the vessels are destroyed by the action of the fungus, as is evidenced by the isolation of the vessels when seen in tangential view. The middle lamellae in the large medullary rays are often dissolved, and the walls of the ray cells show yellowish to blue when treated with chloriodide of zinc. Many of the ray cells and tracheids stain purple, showing delignification.

The bordered pits of the tracheids are uniformly corroded into large round holes which often coalesce, causing the cell to rupture under stress along this line of weakness. The tangential walls of both large and small medullary rays are much perforated, with the holes often uniting. The cells in some of the large rays are entirely destroyed.

Hyphae are found in the cavities of the vessels, and yet their walls and pits are apparently not attacked. Many of the vessels are filled with hyaline hyphae two microns or less in size. Hyphae can also be seen permeating the tracheids, medullary rays, and other elements, running both longitudinally and transversely through them.—W. H. LONG, Forest Pathology, Bureau of Plant Industry, Albuquerque, N. Mex.

Polyporus dryadeus, a root parasite on white fir.—While on field work in what is known as Tejano Canyon in the Sandia Mountains near Albuquerque, New Mexico, the writer found many dead and dying trees of

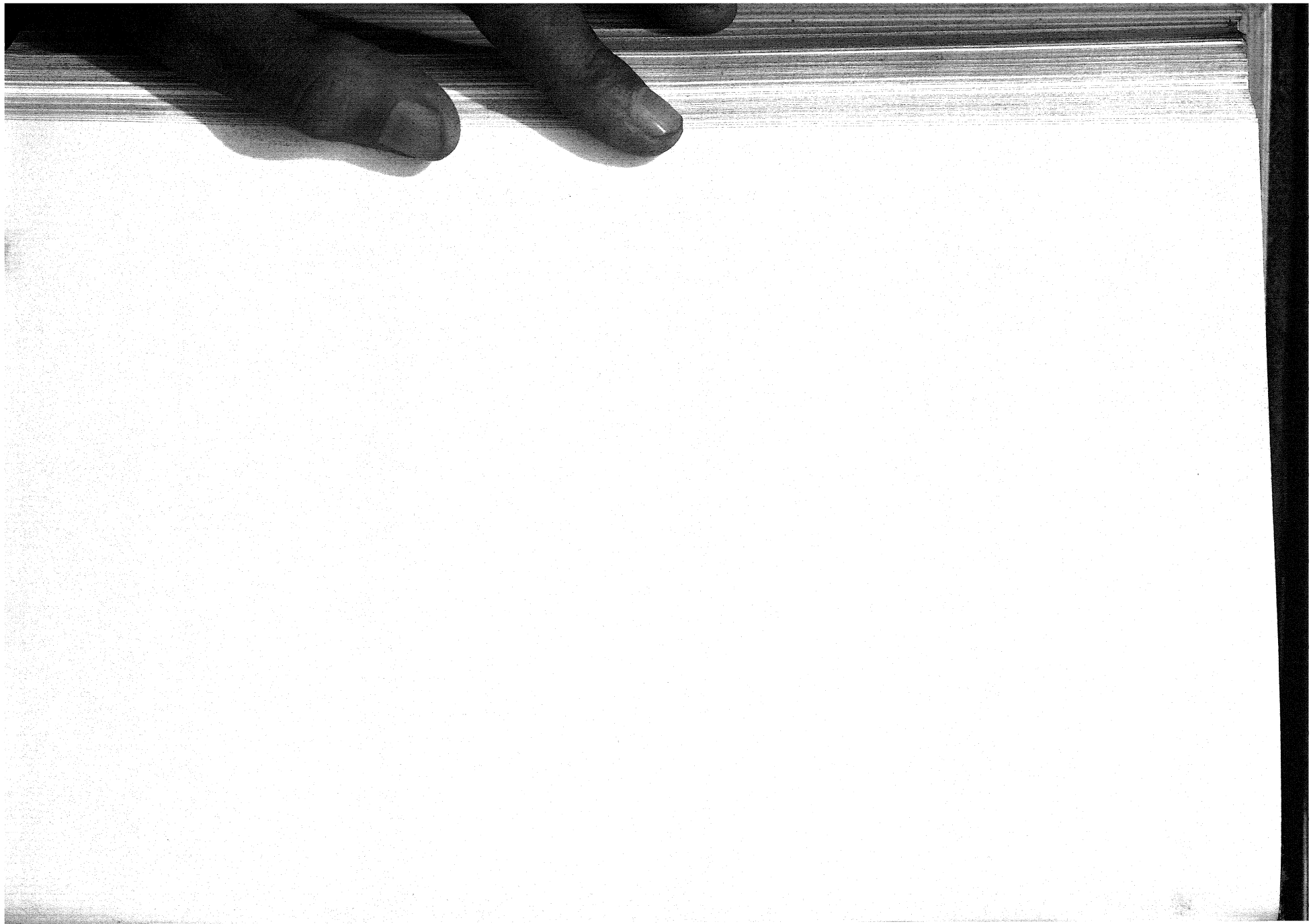
white fir, *Abies concolor* Lindl. & Gord. Some of the trees were more than two feet in diameter, while others were small saplings. An examination of the diseased trees showed them attacked by some root parasite. Trees were found in all stages of the disease. Many were still alive but in a dying condition. In such cases all the larger roots were dead and in a rotten condition, while a few of the smaller surface roots were still alive but with their tips dead, and the disease was progressing along the roots toward the base of the tree. On some of the larger trees, sporophores were found attached to the collar of the tree. Old as well as fresh ones were found, some even in the sporulating stage. Specimens of various stages of the rot and of the sporophores were collected for study. These specimens agreed with *Polyporus dryadeus* in all essential characters, both gross and microscopic.

In a previous paper[1] the writer described this fungus as it occurs on oak and also called attention to a collection from the State of Washington on *Tsuga heterophylla* (Raf.) Sarg. It is now reported from another conifer, white fir. A collection has also been seen from California on this last host.

The old sporophores on white fir have the hymenium with tubes splitting into irregular areas. The surface of the pileus, in weathering, splits into irregular polygonal scale-like areas that separate slightly from each other. The fission is more or less parallel to the surface of the pileus. The weathered pileus is dark brown to almost black. The pileus of the fresh sporophores is typical of the eastern specimens of this fungus as it occurs on oak.

The setae were cat-claw shape, dark chestnut brown, thick-wall, 10 to 12 microns thick at the base by 25 to 30 microns long, averaging 11.5 by 26 microns. The spores were hyaline, obovate to globose, ranging from 7–8.5 by 7.5 to 10 microns, average 7.25 by 8.25 microns, usual size 7 by 8.5 microns. These characters of setae and spores agree with those found on oak.—W. H. LONG, Forest Pathology, Bureau of Plant Industry, Albuquerque, N. Mex.

[1] Long, W. H. *Polyporus dryadeus*, a root parasite on the oak. Jour. Agr. Res. 1: 239–250. 1913.



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STUDIES ON THE OVERWINTERING OF PHYMATOTRICHUM ROOT ROT[1]

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INTRODUCTION

Though the cotton-root-rot disease, caused by *Phymatotrichum omnivorum* (Shear) Duggar, has been studied since 1888 (6), the development of successful control methods has been impeded by incomplete knowledge of the life history of the organism. The present paper includes the results of some studies on the means by which the fungus overwinters. In another paper (10), we have summarized experiments which indicated that the fungus spreads from plant to plant during the growing season, along the roots, which are the known avenues of spread. It appears from this previous work and the experiments reported below that living infected roots and sclerotia or dormant strands are the important means by which the fungus overwinters. So far as we know, it probably does not survive in the soil in a vegetative condition independently of roots.

I. OVERWINTERING ON INFECTED ROOTS

It has already been indicated (11) that infected live roots of susceptible hosts offer a ready means for the overwintering of root rot. Casual observers, even planters who have grown cotton for over forty years, have considered that, since the tops of the plants always succumb to the first killing frost in the fall, the roots are killed by the frost also. We have found that, although the tops of cotton plants are killed by frost, many of the roots of such plants remain alive through the winter. The root-rot fungus hibernates on roots apparently by gradually spreading during fall, winter, and spring and infecting the parts of the roots that were still normal at the time of frost.

The results presented below support the conclusion that *P. omnivorum* remains viable on infected roots in the ground only so long as such roots remain at least somewhat alive and apparently only in the vicinity of

[1] Paper read at the Des Moines meeting of the American Phytopathological Society and published with the approval of the Director as Technical Contribution No. 95 of the Texas Agricultural Experiment Station.

the living tissue. In these experiments, we have studied by various methods the viability of the fungus on roots of varying stages of decay. Such infected roots, to be tested in cultures as inoculum or in other ways, were in general classified or described as "living" or "decayed." We have classified as living all those infected roots with definite areas of live tissue irrespective of the amount present and including all stages from completely normal roots to roots nearly decayed but with occasional streaks of remaining live tissue. All other infected roots were classified as decayed, a group which therefore included roots with all tissues dead and also the occasional instances in which it was difficult to tell whether all the tissues were actually dead. Thus, infected roots were classified as living only when there was no doubt that such roots, though partly invaded by the root-rot fungus, contained large or small areas of living tissue; while the roots classified as decayed included those entirely killed by the fungus and also some roots in which, though the greater portion of the tissues was dead, there remained doubtful traces of live tissue.

Most cotton roots infected with root rot are readily grouped into the classes defined above because of the characteristic visible demarcation between the root-rot lesions and the noninvaded portions. The diseased areas are dark, softened, and sharply depressed; while the sound, noninvaded areas retain their normal color and turgidity. After the disease has involved the entire surface of the roots, there remains for a time some living tissue within the central cylinder. It was therefore necessary to cut into roots whose entire surfaces were decayed to find whether the decay was perhaps superficial only or extended to the center. This was done sometimes by sectioning with a knife or clippers or very frequently by thrusting the thumb nail into the tissues. By this test, we found it simple to differentiate the turgid, live tissues from the disorganized and softened decayed tissues. As noted above, however, doubtful infected roots were classified as decayed.

Winter survival of normal cotton roots.—In the springs of 1925, 1926, and 1928, taproots of normal cotton plants were pulled out to ascertain the condition of the roots after the winter. Table 1 shows that not only the taproots but also the laterals, and even many of the rootlets (Fig. 1, a and b) of the plants, had survived the winter, whether the plants were plowed under or left undisturbed. The viability of these roots was determined by visual examination, by cutting into the epidermis and cambium, and also by sprouting the roots in water or sand. Lateral roots survived in large numbers, probably because they were more deep-seated and therefore less likely to be disturbed by the plow.

Infection on overwintered roots.—The winter survival of taproots and lateral roots of cotton is significant here because such roots are frequently

TABLE 1.—*Overwintering of normal cotton roots, as shown by excavation and examination of roots in fields planted to cotton the previous season*

Date and location		Previous field treatment	Number of plants examined	Percentage of roots alive		
				Tap-roots	Large laterals	Fine laterals
1925						
March 7	Tyler	Undisturbed	200	80	85	49
"	"	Plowed under Nov., 1924	160	82	73	32
April 4	Brownsville	Undisturbed	146	100	98	92
"	"	Plowed under Nov., 1924	300	95	90	84
April 6	Temple	Undisturbed	500	83	70	41
"	"	Plowed under Dec., 1924	260	82	72	40
1926						
March 6	San Antonio	Undisturbed	600	96	93	92
"	"	Plowed under Dec., 1925	320	90	86	70
March 8	Laredo	Undisturbed	260	91	90	83
"	"	Plowed under Dec., 1925	400	91	87	74
March 17	Dallas	Undisturbed	300	80	73	65
"	"	Plowed under Jan., 1926	400	60	51	45
March 22	Wichita Falls	Undisturbed	259	60	53	47
"	"	Plowed under Jan., 1926	365	58	39	23
March 29	Balmorhea	Undisturbed	390	48	20	20
"	"	Plowed under Dec., 1926	451	40	23	19
1928						
March 6	College Station	Plowed under Oct., 1927	96	86	60	42
April 4	San Benito	Plowed under Sept., 1927	382	100	94	90
April 12	San Antonio	Plowed under Nov., 1927	465	91	80	71
April 14	Dilley	Plowed under Oct., 1927	260	100	91	68
April 16	Laredo	Plowed under Oct., 1927	325	92	100	100

infected with root rot. In table 2, observations are presented which show the frequent occurrence of root rot on the roots of cotton plants adjoining root-rot spots. Some of the plants found infected here doubtless were infected in fall, while others probably were infected as the result of winter spread, discussed below. Root-rot infection has similarly been found on the overwintered roots of many other perennial and biennial plants, non-cultivated as well as cultivated.

Viability of P. omnivorum on overwintered roots.—Cultures were made in attempts to isolate the fungus from infected material collected at different times of the year. The method of culturing was that previously described (8). The organism was readily isolated from cotton roots collected during the fall, winter, and spring months, though it will be noted (Table 3) that the percentage of pure cultures obtained diminished as the

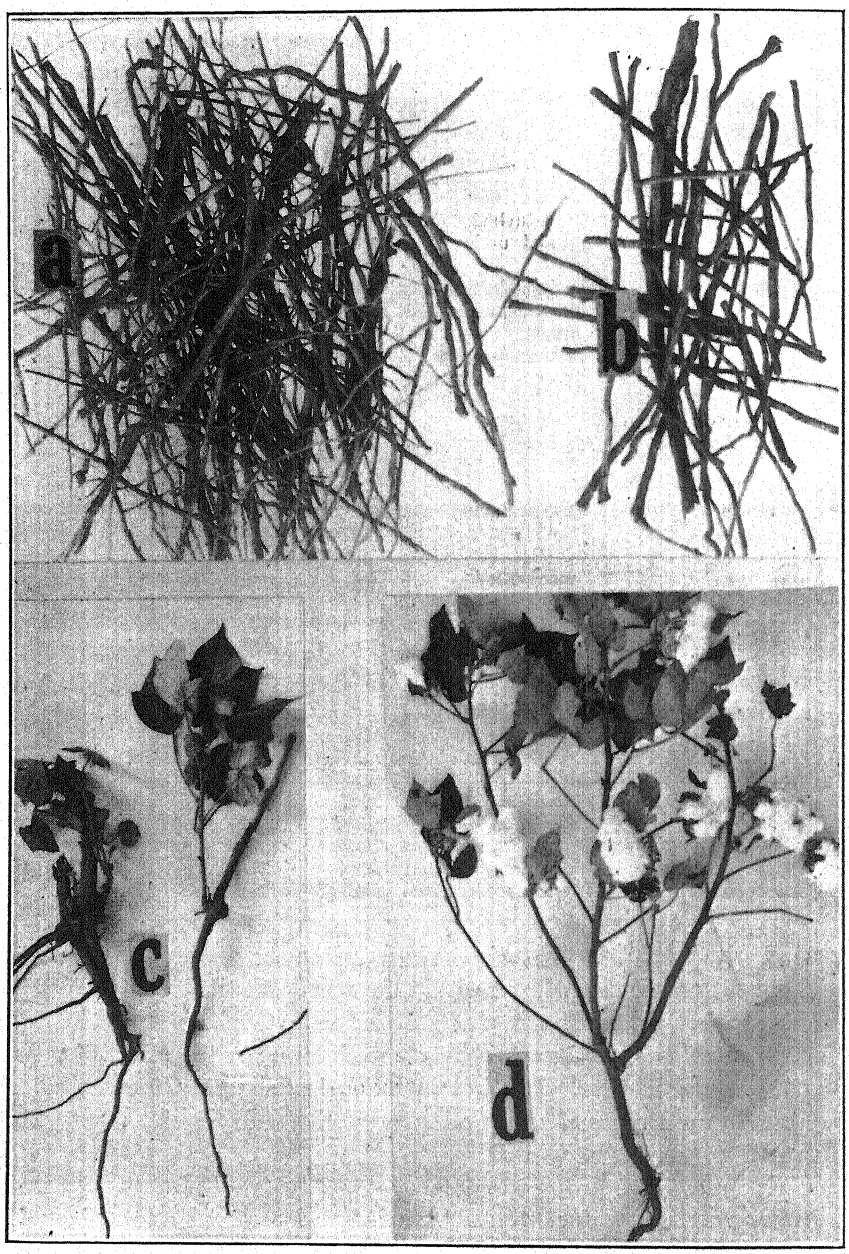


FIG. 1, a and b: Overwintered cotton roots found April, 1928, in a single excavation in a field at College Station; a, live roots; and b, dead roots. c and d: New growth from cotton roots overwintered in oat stubble field, Robertson County; c, sprouts from the overwintered roots; and d, mature plant, with cotton bolls, developed from overwintered root.

TABLE 2.—*Root-rot infection found in periodic field examinations of the living roots of cotton plants pulled from slightly beyond the periphery of root-rot spots*

Date and location		Number of plants examined	Percentage of plants with infected roots
Sept., 1926	College Station	46	12
"	Bryan	200	9
"	Benchley	79	30
"	Temple	300	28
Oct., 1926	College Station	160	21
"	Bryan	90	19
"	Benchley	150	17
"	Temple	300	24
Nov., 1926	College Station	25	16
"	Bryan	100	19
"	Benchley	100	21
Dec., 1926	College Station	100	14
"	Temple	300	17
Jan., 1927	College Station	100	9
"	Benchley	400	13
"	Temple	500	17
Feb., 1927	College Station	90	7
"	Temple	300	11
March, 1927	Bryan	150	9
"	Benchley	200	11
"	Temple	500	12
April, 1927	Bryan	100	6
"	Temple	200	9
May, 1927	Bryan	60	5
"	Temple	300	7

age of the material increased. The fungus was readily isolated from infected, living roots but not from roots which were infected but decayed.

Viability of P. omnivorum as compared with Fusarium vasinfectum, on dried roots.—Isolations have been reported previously (Texas Sta. Bul. 307, Tables 25 and 26) from infected cotton roots which were dried indoors and outdoors. Phymatotrichum could be obtained only from roots dried no more than twenty days. In contrast, isolations have been made from cotton plants infected with Fusarium wilt (*Fusarium vasinfectum*) which were exposed to air drying for various intervals. The results (Table 4) indicate that the Fusarium of cotton wilt, unlike *P. omnivorum,* is readily isolated from different parts of infected plants which have been dried for more than a year. We have also been able to isolate *F. vasinfectum* from

TABLE 3.—*Results of attempted isolations of Phymatotrichum omnivorum from living though infected cotton roots and from infected, decayed cotton roots*

Source of material	Date of culturing	Results with infected, living roots		Results with infected, decayed roots	
		Number of tubes	Percentage of tubes yielding pure cultures of *P. omnivorum*	Number of tubes	Percentage of tubes yielding pure cultures of *P. omnivorum*
Zemanek farm, Benchley, Texas	Aug. 16, 1926	90	12	45	0
	Sept. 22, 1926	111	7	316	0
	Oct. 18, 1926	390	6	316	0
	Dec. 14, 1926	518	4	260	0
	Jan. 16, 1927	400	2	100	0
Williams farm, Benchley, Texas	Aug. 18, 1926	210	11	210	0
	Sept. 24, 1926	464	6	209	0
	Oct. 20, 1926	416	5	210	0
	Dec. 20, 1926	414	3	270	0
	Jan. 18, 1927	200	2	87	0
	March 20, 1927	119	1	52	0
	Feb. 23, 1928	523	2	189	0
	March 22, 1928	427	1	136	0

infected cotton plants which had succumbed to the disease and overwintered in the field, while, as noted above, we have not been able to obtain *P. omnivorum* from cotton roots infected by root rot and decayed.

TABLE 4.—*Isolations of Fusarium vasinfectum from infected cotton plants exposed to air drying, beginning July 14, 1923*

Date of culturing	Age of material in days	Average number of Fusarium colonies per plate in culture from[a]		
		Main stem	Primary branches	Taproot
Sept. 12, 1923	60	25	23	.....
Oct. 11, 1923	89	.....	.....	29
Oct. 31, 1923	109	33	.....	32
Jan. 17, 1924	187	16	21	.....
Jan. 30, 1924	200	.....	.....	24
Feb. 15, 1924	216	9	.....	11
March 20, 1924	250	6	.....	9
July 17, 1924	369	2	.....	5

[a] The number of plates per series varied from 12 to 24.

Viability of P. omnivorum on infected live roots.—The isolation results in table 3 indicated that the root-rot fungus overwinters in an active form on live, infected roots but not on decayed roots. The following experiments, planned partly with the idea of developing a rapid method of determining whether the fungus was viable in roots of various stages of decay, have furnished additional information on this point. The first method tried consisted of collecting infected cotton roots, wrapping them in moist cloth, and placing these bundles of roots and cloth in various containers. Freshly infected living roots were compared by this method with older and with decayed roots. The results are given in table 5. With

TABLE 5.—*Relative production of new strands of Phymatotrichum omnivorum from living though infected cotton roots and from infected, decayed cotton roots; roots placed in moist chambers the day collected*

Root material		Treatment	Results after three weeks; development of *P. omnivorum* strands
Type	Number of roots and date collected		
Live, freshly infected cotton roots	23 roots, Dec. 11, 1927	Wrapped in moist cheesecloth and placed under covered bell jar	Profuse growth on 2 roots; fair growth on 19; scant growth on 2 roots
"	15 roots, Dec. 11, 1927	Placed in quart Mason jars two-thirds full of water, 3 roots per jar, 5 jars in all	Eight roots produced copious strand growth on the surface of the water. None on submerged area of any roots
Infected and decayed cotton roots	30 roots, Dec. 11, 1927	In quart Mason jars as above, except 5 roots per jar and 6 Mason jars in all	No growth
Live, freshly infected cotton roots	60 roots, Dec. 27, 1927	Wrapped in moist cheesecloth and placed in covered glazed crock	Profuse growth on 10 roots; fair growth on 10 roots; scant growth on 40 roots
"	100 roots, Dec. 28, 1927	"	Profuse growth on 60 roots; fair growth on 23 roots; scant growth on 17 roots
Infected and decayed cotton roots	1500 roots, Dec. 27, 1927	"	No growth

live, freshly infected cotton roots, the fungus grew out from the roots so abundantly that it was noticeable to the naked eye as well as microscopically. This occurred whether the roots were placed in moist chambers or in quart Mason jars with water. On the other hand, the roots that were

infected but decayed failed to yield any growth of *P. omnivorum* under the same conditions.

The question now arose whether the profuse growth of the root-rot fungus obtained from the cotton roots in moist chambers would be able to infect a susceptible host. To determine this, sound turnip roots were placed in moist chambers with live, freshly infected cotton roots; and other turnips were placed in contact with infected but now decayed cotton roots. Each series was set up within eighteen hours of the time the cotton roots were collected, and the roots were kept moist continuously until used. The results are shown in table 6. The sound turnip roots placed in con-

TABLE 6.—*Tests of the virulence of Phymatotrichum omnivorum from cotton roots to turnips, by wrapping in moist cheesecloth 12 sound turnips with each 100 cotton roots and placing the bundles in moist chambers*

Inoculum	Results after four weeks	
	Root-rot strands present on cotton-root inoculum	Percentage of turnip roots infected with root rot
Check. Live, normal cotton roots; 500 roots collected Oct. 25, 1927	None	0[a]
Live, infected cotton roots; 300 roots collected Nov. 1, 1927	Strands profuse on 60 per cent of roots; fair amount on 20 per cent; scant on 14 per cent; and absent on 6 per cent	68
Live, infected cotton roots; 500 roots collected Nov. 2, 1927	Strands profuse on 48 per cent of roots; fair amount on 50 per cent; and scant on 2 per cent	52
Infected, decayed cotton roots; 2,000 roots collected Nov. 5, 1927	None	0

[a] Turnips sound and sprouting.

tact with live, freshly infected cotton roots succumbed to root rot; while none of the turnips mixed with sound cotton roots or with infected and decayed roots were attacked.

AGE-OF-INOCULUM EXPERIMENT

To determine further whether the fungus *P. omnivorum* remains viable in the tissues of infected cotton roots after such roots are dead, inoculations of cotton plants were undertaken with inoculum consisting of the

roots of cotton plants which had succumbed to root rot at various intervals preceding the date of inoculation (Table 7). It has already been proved (8) that the roots from plants recently wilted from root rot can be used to inoculate healthy plants.

During the season of 1928, cotton plants in the experimental field at College Station were inspected daily for the first above-ground symptoms of root rot. Those plants that showed the characteristic sudden wilting were marked with a label that indicated the date. There were, therefore, available in September plants that had succumbed to rot rot quite recently

TABLE 7.—*Inoculations of normal cotton plants with root inoculum from infected cotton plants, pulled at varying intervals after the plants wilted from root rot. Each lot of inoculum included 20 taproots and was used to inoculate at least 20 cotton plants*

Lot No.	Description of inoculum pulled, and used in inoculations, September 13, 1928		Results of inoculations; percentage of plants infected with root rot, November 2, 1928
	Days since plants wilted from root rot	Abundance of Phymatotrichum strands on roots	
1	1	+++	92
2	7	+++	100
3	14	++	43
4	21	+	0
5	28	tr	0
6	35	tr	0
7	42	tr	0
8	49	tr	0
9	56	tr	0
10	63	tr	0

and had tops still green though wilted; other plants attacked during the middle of the season, with tops now dead but roots still alive; and, finally, plants that were attacked early in the season, with tops quite dried and roots almost completely disintegrated. Twenty plants were selected on September 13 for each of ten lots of inoculum. The intervals since the dates of wilting varied from one day for Lot 1 to seven, fourteen, etc., days by weekly intervals, to Lot 10 which consisted of roots from plants that had wilted nine weeks previously. The roots were removed from the ground after the soil around them had been loosened and care was taken not to lose the nearly disintegrated lower portions of the taproots of the more severely injured plants. In every case, however, the inoculum consisted of only the upper foot of the taproots rather than of the entire root systems.

Notes were taken on the state of preservation of these roots as pulled from the ground, before using them for inoculum in the inoculation series summarized in table 7. The more recently wilted plants had much the better-preserved roots. In Lot 1, the lesions had girdled the taproots but had involved only a small part of the tissues, most of which were still alive; and there was heavy mycelial growth over the surface of the roots. In Lot 2, the lower portion of the diseased areas had begun to disintegrate and the fungus strands there were deteriorating, although the upper portions of all the roots were still alive and bore the thick, fibrous strands typical of *P. omnivorum*. In Lot 3, the disease had progressed farther and involved the greater portion of each taproot, but each root had still considerable living tissue above the decayed area. In Lot 4, the surfaces of the roots were disintegrated and frayed; and the fungus threads were scarce, adhered now only loosely, and had lost their normal color. Apparently, the only tissues still living were those at the surface of the ground, and in four of the twenty roots even this portion appeared dead. In the root inoculum of Lots 5 to 10 there were further progressive decay of the roots and deterioration of the fungus. Living tissue in these roots was confined to the extreme top of the taproots, and the number of roots in which even this restricted region remained alive decreased progressively to 2 roots in Lot 9 and to none in Lot 10.

These selected taproots were used to inoculate large, normal cotton plants growing in metal cans of surface soil material of Lufkin fine sandy loam. The cans were twenty inches tall and fifteen and a half inches in diameter and contained about ten cotton plants, each. One of the pieces of cotton-root inoculum was used for each plant or occasionally for two plants. The plants were inoculated on September 13, 1928; brought into the laboratory October 30; and final notes were taken on the development of root rot on November 2, 1928.

As may be noted from table 7, there was in this experiment a definite relation of the interval between wilting of plants and the date when roots were used as inocula with the ability of this root inoculum to transmit root rot. Of twenty-five plants inoculated with roots from plants wilted the previous day, twenty-three became infected with root rot. All of the thirty-four plants inoculated with the week-old inoculum were infected, while only ten of twenty-three plants inoculated with two-week-old inoculum showed symptoms of root rot. Of the one hundred and twenty-seven cotton plants that were inoculated with taproot inoculum from plants that had wilted still earlier in the season, not one was attacked by the disease.

The fungus appears to have been virulent on the taproots of freshly infected plants for a period of between two and three week after the tops

of the plants had wilted. Further experiments during the same season (3) indicated that the interval between infection and wilting is often two to three weeks also. The total time between infection of roots of the cotton plants and the time when the fungus was no longer virulent on the upper part of the taproots was probably between four and six weeks.

It appears from this experiment that the root-rot fungus survived in an actively virulent condition only so long as some of the host tissues remained undecayed. It should be emphasized that the specific time of survival found here applies only to the particular seasonal and soil conditions of this experiment. Furthermore, it is to be remembered that only the upper foot of taproots was used as inoculum. This region is usually the first to be decayed, and, after the upper parts of the roots are dead, the root-rot fungus may still be spreading actively along lower roots which were infected later. The chain of events which includes infection, decay of the roots, and death of the root-rot fungus must of course occur much more slowly in winter.

PHYMATOTRICHUM OMNIVORUM ON THE LIVING PARTS OF ROOTS

Results of previous experiments (Tables 3, 5, 6 7), particularly in the isolation of cultures and the use of roots of diseased plants as inoculum in inoculation experiments, suggested that even on the roots of plants attacked by root rot the organism is usually present in a living condition only near parts of the roots as yet undecayed. Further test of this point was possible by a method in which portions of infected roots are thrust part way into moist, unsterilized sand in a moist chamber. In such soil chambers the root-rot fungus develops profusely from infected, live roots. This method appears quite valuable for determining the viability of Phymatotrichum on infected roots. On culture media, saprophytic organisms present in the rotted portions of the roots may smother and obscure Phymatotrichum growth. With the soil chamber method, on the other hand, while for a few days there may be a superficial development of saprophytic organisms, notably Rhizopus, this early, fluffy growth soon vanishes. The more slowly growing Phymatotrichum strands do not appear to be affected by this superficial contamination but grow at approximately the same rate and to about the same distance over the soil in the contaminated as in the uncontaminated chambers.

A preliminary series was started December 11, 1926, with a cotton plant collected in our experimental field at College Station. This plant had been inoculated with root rot some months earlier and only the upper part of the taproot was now alive. The entire root, from the dead, decorticated tip up to the normal part above the root-rot area, was sliced into pieces two cm. long, each of which was thrust into moist Norfolk sand in a pint

Mason jar. In ten days there was a profuse development of Phymatotrichum strands from the two pieces from near the junction of rotted and normal regions. One of these pieces had a live central cylinder with only the cortex rotted, and in the other piece not only the central cylinder but also part of the cortex was still alive. No root-rot strands developed from the lower portions of the root, where it was completely decayed, nor from the normal piece from above the diseased area.

On January 28, 1929, the taproots of six cotton plants of the 1928 crop were pulled from the edge of a root-rot spot in a field at College Station. These were naturally infected plants. The roots were found to

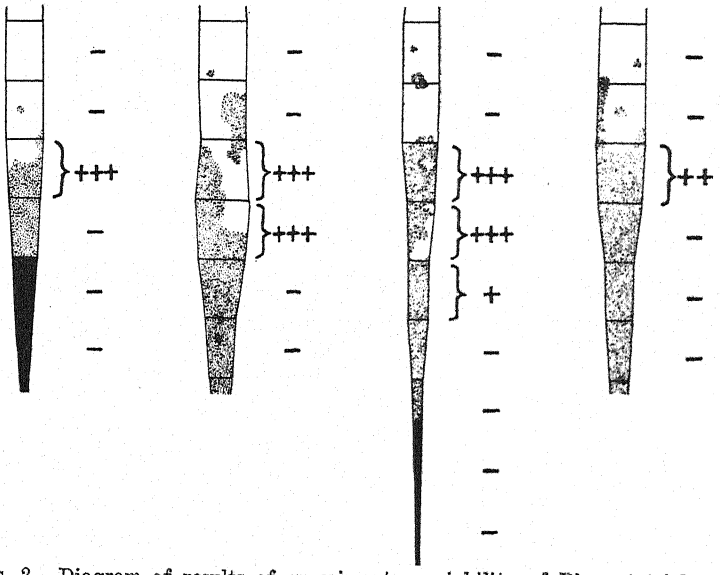


FIG. 2. Diagram of results of experiment on viability of Phymatotrichum in the living and dead parts of roots. In diagram, white areas represent portions of roots with cortex and wood alive; shaded parts, cortex decayed but woody cylinder alive; black areas, cortex and wood decayed. Roots cut into 2 cm. portions as indicated by horizontal lines; growth of strands from these portions when placed in soil chambers indicated by +, and no growth by –.

be at least partly alive, although all of them were definitely affected by root rot. They were taken to the laboratory, lateral roots were clipped off, and the taproots were washed gently in distilled water. Each root was cut into short pieces about two cm. long. These pieces were placed in moist sand in separate Mason jars which were then covered with inverted petri dishes. Observations on possible growth from the pieces were continued for five weeks. The results are presented graphically in figure 2, which, however, includes only the four roots from which some Phymatotrichum growth was secured. Profuse Phymatotrichum strands de-

veloped from sections of three of these roots and somewhat less extensive growth from one of the sections from the fourth. It is to be noted that in no case did growth come from the completely decayed portions of the roots. It was always from the sections at the junction of the normal and diseased areas. Six of the seven sections from which root-rot strands developed included both normal and diseased cortical tissues. In the seventh section the cortex was rotted but the central cylinder was still sound and was contiguous to normal cortical tissue.

These results indicate that *P. omnivorum* survives in an active condition only near the yet living portions of roots that are attacked by root rot rather than on the decayed portions of the same roots.

WINTER SPREAD OF ROOT ROT

As we have found that the root-rot fungus is active on live, infected cotton roots during the winter months, it was of interest to determine whether the fungus might also continue to spread along the roots after the tops of the plants are killed by frost.

Two cotton fields in the vicinity of College Station were selected in the fall of 1927 for these studies. A single large isolated root-rot spot in one field and two well-separated spots in the second field offered the opportunity of following spread that might occur. Initial notes were taken on September 30, when first all plants dead or dying from root rot were pulled and then the plants along each row were pulled until all those with taproots definitely infected had been removed. It was considered that the limit of the root-rot zone at the time was established when the final plant pulled had completely normal roots. The zones thus established (shown by solid lines in figure 3) included presumably all the plants infected during the summer and up to September 30.

Later observations were made October 27, November 30, December 20, January 22, and February 20. Notes for the two later dates could not be secured for one of the fields of figure 3, a, in which the cotton was plowed under early in January. At each date, cotton plants were pulled around the entire periphery of the spots until the limits of root infection were again established. The original area of the spots and the distances of spread were measured and are shown graphically in figure 3, a, b, c.

It is evident from these preliminary results that considerable spread of root rot occurred even during winter along these cotton roots. The longest spread appeared to have been in Row 10, figure 3, c. Here infection advanced twelve feet along the row between September 30, 1927, and February 20, 1928. It is to be noted that in the next row, 11, there was little advance of root rot during the winter. Similarly, in the other spot

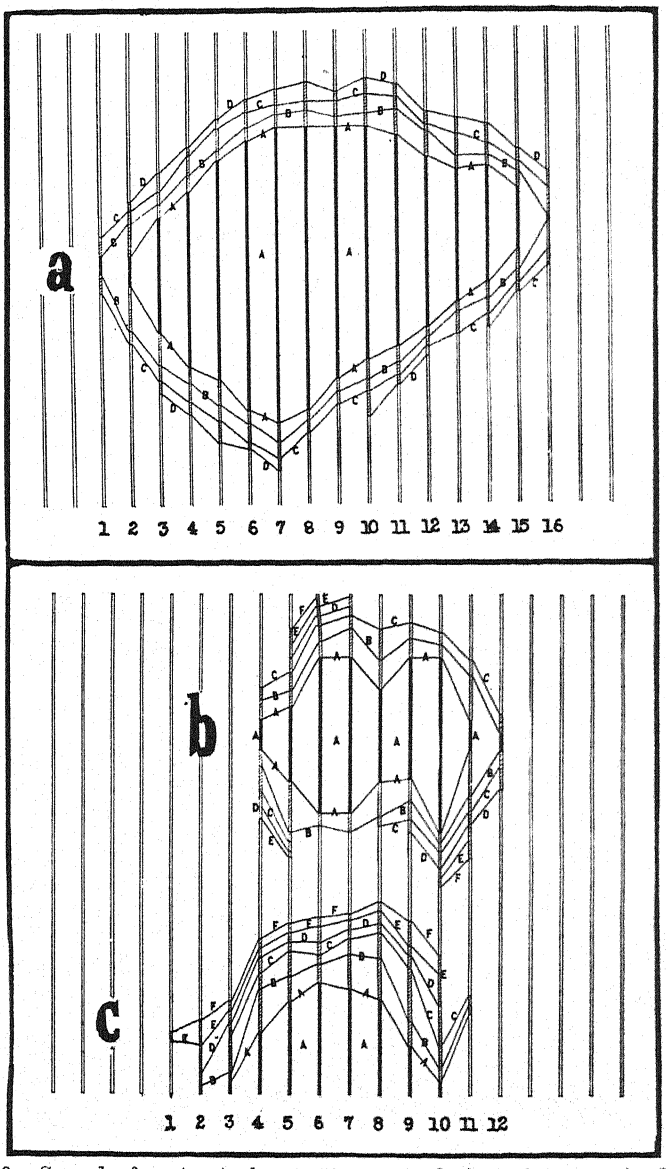


FIG. 3. Spread of root rot along cotton roots during winter; a, in field with a single large root-rot spot; b and c, in another field with two root-rot spots. Solid lines and areas included by line A indicate limits of infection on the roots on September 30, 1927; spread along roots during winter to B, by October 27; to C, by November 30; to D, by December 20; to E, by January 22; and to F, by February 20.

in this field (Fig. 3, b), spread in the upper part of Rows 4 and 5 was much more rapid than in Rows 2 and 3 during the first part of the winter; while, during January and February, spread continued in Rows 2 and 3 but ceased in Rows 5 and 6. These results indicate not only the extension of root-rot spots during winter but also seem to explain in part the apparently "erratic" reappearance of root-rot spots, which has been mentioned by many writers. These results further suggest that survival of *P. omnivorum* in an active vegetative condition during the winter months is the result of gradual spread of the fungus to previously normal roots or portions of roots.

Relation of weeds to overwintering of root rot.—Farmers often sow oats or wheat between the rows of cotton plants as soon as the cotton has been harvested. The land is usually not plowed, the grain being drilled

TABLE 8.—*Cotton roots, weeds*[a]*, and root rot on weeds found in stubble of 1927 oat field, that was in continuous cotton 1919–1927 (Cavitt Ranch, Robertson County, Texas)*

	Date of excavation	Percentage of live, overwintered taproots	Percentage of live, overwintered lateral roots
Live, overwintered cotton roots found in 6 excavations, 6 ft. x 6 ft. x 3 ft., at each date indicated	March 2, 1928	82	73
	May 2, 1928	42	21
	August 2, 1928	4	0
	September 1, 1928	1[b]	0

[a] The plants listed here were identified by Prof. H. Ness, late Botanist of the Texas Agricultural Experiment Station.

[b] The 1927 roots still alive on September 1, 1928, had all sprouted before this date and matured a crop.

	Names of weeds	Percentage root rot
Weeds growing in oat stubble and affected by root rot, September 1, 1928	*Sesban macrocarpa*	40
	Xanthium sp.	21
	Croton monanthogynus	17
	Sida spinosa	14
	Euphorbia humistrata	12
	Croton capitatus	7
	Aster exilis	7
	Eupatorium serotinum	6
	Iva ciliata	4
	Leptilon canadense	4
	Heterotheca subaxillaris	3
	Amphiachyris dracunculoides	3
	Jacquemontia tamnifolia	2
	Ambrosia psilostachya	2

in between the rows of cotton. As shown in table 8, this practice favors the overwintering of cotton roots (Fig. 1, c and d) and also affords a good opportunity for root rot to survive and to be transmitted to the numerous susceptible weeds which invariably appear on stubble land. While oats or wheat may be valuable in a rotation system, the stubble land should be plowed after the grain crop has been harvested and weeds kept down thereafter, if the rotation is intended to reduce root-rot damage to future crops.

Cotton and alfalfa growers in Texas have often noticed root rot appearing in the first crop on newly cleared land. Excavations were made to determine the source of infection in such fields, and the results are summarized in table 1 of Texas Bulletin 393 (9). Early infections in newly cleared fields planted to cotton were traced to infected native vegetation which persisted in the cleared land. Sclerotia may also have been present since they were not looked for in the excavations.

It is not uncommon to find roots of various perennials, particularly shrubs and trees, persisting in cleared land which has been cropped for years. The roots of such plants continue to sprout year after year, in spite of attempts to destroy them with the plow. This is the case with oaks, pecans, and native species of walnuts, all of which are susceptible to root rot. In excavations made at Lancaster, Texas, to determine sources of early infection in cotton, several pecan sprouts were found from stumps, which, according to the records of the owner, had sprouted every year for the last forty years. When excavated, many old root-rot lesions, in some cases healed over, were found on the pecan roots, and there were also lesions of more recent origin, together with strands of *P. omnivorum*. As will be discussed below, large numbers of sclerotia were also found in this field.

The studies above help to explain the persistence of root-rot in many fields. Cotton roots as well as those of many perennial weeds persist in the soil, even though the tops are plowed under during the fall or winter months, as uprooting the taproots and turning them under again do not necessarily kill them. The deeper roots are not reached by the plow and are probably even more important than the superficial roots in the overwintering of the fungus.

Inoculation of normal cotton plants with overwintered, infected cotton roots.—In order to demonstrate the virulence of *P. omnivorum* from overwintered, infected cotton roots, normal plants grown in the greenhouse were inoculated with root inoculum that had actually overwintered, undisturbed, in the field. This inoculum was secured in the lower Rio Grande Valley at Weslaco, Texas, and was pulled in a field of overwintered cotton

in which many of the plants had already sprouted and developed considerable new growth. Root rot had been present in this field during the season of 1928, and we found root rot on many of the plants on March 13, 1929. Infected taproots from overwintered cotton plants were taken to College Station and used to inoculate cotton plants growing in two large metal containers in the greenhouse. Although no wilting occurred in one container, some of the roots were found to be infected when the plants were removed at the end of the season; and all six of the plants in the other container became infected and died from root rot.

From the observations and experiments reported above, it is evident that one of the important ways in which *P. omnivorum* survives the winter is in an actively growing condition on infected but still living roots. The large number of infected but undecayed roots remaining in the ground no doubt offers an important source for early infections of the new crop.

II. SCLEROTIA AS MEANS OF OVERWINTERING

Early in the spring of 1929, numerous excavations were made, in cooperation with H. E. Rea, at San Antonio, Lancaster, Itasca, and Rockwall, to find the source of early infections in fields of cotton. In each place mentioned, the cotton was on land kept in clean fallow during 1928 but in cotton infected with root rot in 1927. With the exception of the Lancaster field, the excavations in 1929 showed that the fields were freed from most, if not all, of the roots of root-rot carriers. However, large numbers of sclerotia were found in excavation of the plants infected in 1929. This was particularly the case at Rockwall and Lancaster, where thousands of sclerotia were found under a single infected plant. It is probable that the sclerotia were produced on the infected cotton grown in the fields during 1927. Large numbers of sclerotia were also found in infected fields of garden beets, sugar beets, and sweet potatoes in truck gardens in the vicinity of Wichita Falls and Iowa Park.

King and Loomis (4) were the first to discover in pure culture the true sclerotia of the root-rot fungus. In the spring of 1929, Neal (5) found similar sclerotia in cotton fields at Greenville and at San Antonio. In the same spring of 1929, B. F. Dana, S. E. Wolff, and H. Dunlavy, all of the Blackland Substation, found sclerotia under infected cotton plants which grew on land which was kept in clean fallow during 1928. These observations agree with those of Neal and indicate that sclerotia are probably an important means of carry-over of root rot.

In addition to sclerotia, as mentioned above, Dana found what appeared to be dormant strands, separate or intermixed with the sclerotia. Similar strands were found by the writers in excavations at San Antonio, Itasca, and Lancaster, Texas. These strands are smooth and void of the outer

acicular fuzzy growth usually characteristic of strands of *P. omnivorum*. Occasionally, remnants of the fuzzy old acicular growth still remain clinging irregularly to the main body of the strand. When placed in moist chambers, the strands germinated readily at the broken ends, producing hyphae typical of *P. omnivorum*. Under field conditions, germination occurred from the exterior of any part of the strands. These dormant strands apparently function as sclerotia, which for some reason have failed to enlarge and develop into the typical shape. Both the sclerotia and the dormant strands were found at depths of eight to twenty-one inches in the ground.

Development of sclerotia in the laboratory.—King and Loomis (4) were able to develop sclerotia in cultures of *P. omnivorum* on sterilized sand and old cotton stems. Dana (1) found that Phymatotrichum growth from freshly infected carrots in moist soil in Mason jars produced large numbers of sclerotia. The writers have been able to develop sclerotia by using as inoculum pieces of cotton roots freshly infected with root-rot in glass containers filled with moist, unsterilized soil. With the method described above, using cotton or carrots as inoculum, sclerotia were produced in unsterilized surface soil material of Houston black clay, Bell clay, Susquehanna fine sandy loam, and Crockett clay loam. With soil containing 25 per cent moisture on a soil air-dry-weight basis, strands of *P. omnivorum* developed profusely along the walls of the containers in six to ten days. Within sixteen to twenty days, many of the larger hyphae began to swell at various places (Fig. 4), and these swellings matured into true sclerotia. The sclerotia are formed in chains or in compound clusters of irregular shapes. Individual sclerotia may be flattened or otherwise distorted to fit the space in which they are produced but are typically fusoid to ovoid. They are at first creamy to pearly white, then gradually turn darker, assuming finally the deep brown buff color of old strands.

In many of the containers in which carrots infected with root rot were used as inoculum, a profuse growth of *Sclerotium rolfsii* appeared as a contamination. The Sclerotium growth was obtained in jars in which Phymatotrichum strands and sclerotia were present as well as in other jars. The spherical sclerotia typical of *S. rolfsii* were produced in large numbers, and it was of considerable interest to compare these sclerotia with sclerotia of *P. omnivorum* produced under the same conditions. Our method of harvesting sclerotia from containers has involved washing, and it was found that true sclerotia of *P. omnivorum* are invariably heavier than water, while those of *S. rolfsii* are almost always lighter than water and may be separated by floating. It is, of course, easy to distinguish sclerotia of *S. rolfsii* by their smoothness and typically spherical shape. Sclerotia of *P. omnivorum*, on the other hand, are larger, irregular in out-

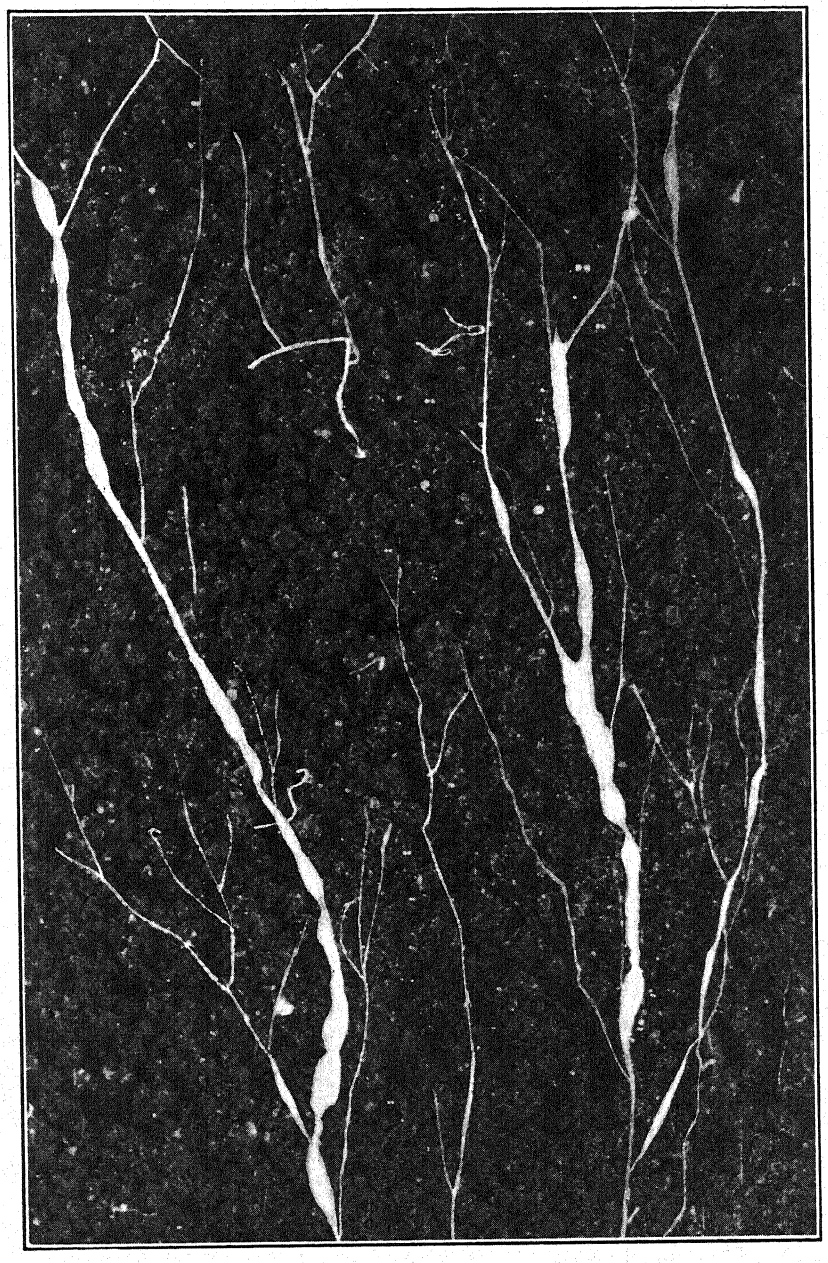


FIG. 4. Formation of sclerotia of *Phymatotrichum omnivorum* as swellings of strands developing in soil chambers. (Retouched photograph, strands, and sclerotia appear lighter-colored than was actually the case.)

line, and, unless mechanically disturbed, are always found in chains or masses or, if single, intercalary as swellings of the main strands.

Sclerotia were also produced in the laboratory at College Station in pure cultures of *P. omnivorum* grown on sterilized cotton stems and also in cultures on synthetic media. It is thus possible to obtain sclerotia of a definite age and from a known source. Results of these studies will be presented later.

Preliminary studies on the germination of sclerotia.—Sclerotia of *P. omnivorum* germinate readily in moist soil, on moist filter paper, or on agar. Germination of the sclerotia takes place at one or both ends of the sclerotium, and the resultant growth is typical of the root-rot fungus.

Three sets of sclerotia were used in one experiment: Sclerotia produced under carrots in a field at the Blackland Substation; some produced from carrot inoculum in glass containers in the laboratory; and a third lot produced from cotton-root inoculum in glass containers in the laboratory. These were washed out separately, placed on moist filter paper, on oatmeal agar, and on moist soil, and incubated in the laboratory at room temperature. All lots germinated rapidly under these conditions, producing yellow, branching strands. These were examined microscopically and found typical of *P. omnivorum*. It was noticed that sclerotia which failed to germinate were soft to the touch and crumbled with slight pressure, while the sclerotia which had germinated were plump and solid and did not crumble under light pressure.

Sclerotia may germinate more than once.—On October 3, 1929, a large number of sclerotia were tested for germination. Many of these produced profuse growth and still showed no evidence of deterioration at the end of the experiment. On October 17, the growth resulting from the previous germination was carefully removed from twenty-one germinated sclerotia, which were then placed again on moist filter paper. By November 4, thirteen of the twenty-one sclerotia had germinated, while the remainder failed to germinate and were found to have softened and in many cases become parasitized by various organisms, particularly *Fusarium* sp., *Phomopsis* sp., and a species of yeast. The growth from the thirteen germinated sclerotia was removed for the second time and the sclerotia placed again on moist filter paper. By December 9, 1929, four of these sclerotia had germinated and the growth was again clipped off. All four of these sclerotia then germinated for the fourth time. On January 3, growth was clipped again from these sclerotia and they were placed on filter paper. One sclerotium germinated the fifth time. The same sclerotia can evidently germinate at least five times under these conditions.

Pure-culture growth from sclerotia.—Sclerotia were germinated and transfers made to sterilized cotton roots and to various other media. The

growth resembled in every respect that obtained with isolations from infected roots. The strain isolated from the sclerotium has been grown on the various media on which stock Phymatotrichum cultures have been grown and is similar in cultural characteristics as well as microscopically. As detailed below, the cultures from the sclerotium as well as growth directly from sclerotia have been proved pathogenic to cotton plants.

Inoculation of normal cotton plants with sclerotia.—The following experiment was carried out to determine whether growth from germinated sclerotia can cause infection of cotton plants. Fresh sclerotia were washed out of soil obtained from a carrot field, dried to remove excess moisture, and placed at the bottom of quart jars, fifty sclerotia to the jar. Normal cotton plants were secured from a field free of root rot and placed in jars, which were filled with sifted surface soil material of Houston black clay to which was added 25 per cent moisture on a dry-weight basis. Pure-culture inoculum was used in other jars, and, finally, two jars were used as checks, which were left uninoculated. The jars were weighed daily and the original soil-moisture content maintained by adding water as required. Three days after the cotton plants were transplanted to the jars, the leaves of all the plants wilted, and within ten days all had shed. New leaves were later produced. When the plants were finally washed out for examination of the roots, clear-cut results were obtained, as shown in table 9. Infection of the roots occurred in every Mason jar where sclerotia were used as inoculum, and good infection also occurred where pure cultures were used. The uninoculated plants remained healthy.

Shortly after the beginning of the experiment, strand growth was observed on the glass walls of the inoculated jars. By November 16, many of the strands in jars a, b, c, d, g, and h of table 9, began to inflate into typical sclerotial forms, and, by November 27, the swellings of the strands had matured into sclerotia. At the end of the experiment, the soil in each Mason jar was washed carefully through a fine sieve and the residue examined for sclerotia. These were found in the jars that had contained inoculum, but, as was to be expected, in none of the uninoculated checks.

This experiment has proved that growth from sclerotia is able to infect normal cotton plants. Sclerotia of *P. omnivorum* probably play an important rôle in the overwintering of the causal organism. Considering the ease with which sclerotia may be produced in the laboratory, they may also serve as a convenient source of inoculum in experiments.

III. SPORE STAGES AS POSSIBLE MEANS OF OVERWINTERING

Phymatotrichum stage.—The Phymatotrichum, conidial stage of the root-rot fungus, was first described by Thornber (12, pp. 160–162) and was again described and named by Duggar (2). This stage appears as

TABLE 9.—*Inoculation experiment with sclerotia of P. omnivorum; inoculum placed on bottom of Mason jars which were filled with moist soil into which cotton plants were transplanted*

Inoculum	Jar	Results after two months	
		Condition of plants	Number of new sclerotia produced
50 Sclerotia per jar	a	One plant with top and taproot sound, but lateral roots infected; other plant, top dead, entire root system infected	124
	b	Both plants, tops dead, entire root systems infected	135
	c	"	140
	d	"	318
	e	"	0
	f	"	0
	g	"	157
Pure culture of *P. omnivorum* grown on sterilized cotton stems	h	One plant with top sound, taproot sound, only rootlets infected; other plant, top dead, entire root system infected	8
None (check)	i	Both plants, tops and root systems sound	0
	j	"	0

spore mats on the surface of the ground, usually around plants which have died from root rot. Spore mats are produced during warm, moist weather in the spring, summer, and early fall months. The writers have demonstrated actual continuity of hyphal growth from the subterranean Ozonium strands, with characteristic acicular branchings, to the conidia-bearing mycelium of Phymatotrichum spore mats aboveground (10): This, together with the fact that Taubenhaus and Killough (11) have found the Phymatotrichum spore stage in pure culture, leaves no doubt that the vegetative or Ozonium stage and the Phymatotrichum conidial stage are actually stages of the same fungus.

As yet, we have obtained only low percentages of germination with the Phymatotrichum spores, and the germ tubes produced soon disintegrate and seem incapable of further growth or infection. We do not know what

becomes of the millions of Phymatotrichum spores which are formed and are scattered by wind or irrigation water. There is no evidence that they can cause infection. We do not know whether the Phymatotrichum spores are a possible means of overwintering.

Possible perfect stage.—Shear (7) found a young sprout of Osage orange (*Maclura aurantiaca* Nutt.) dying from root rot. On the stem of the affected sprout appeared a hymenium with the typical spiny structure of a Hydnum. Believing this to be the perfect stage of the root-rot fungus, he named it *Hydnum omnivorum*. The evidence of a connection of the Hydnum stage with Ozonium, as stated by Shear himself, is only circumstantial. The writers have repeatedly found a Hydnum in cotton fields near College Station and in other parts of the State on the surface of the ground and on cotton plants previously killed by Phymatotrichum root rot. Tissue cultures were made from the spiny sporophores of this Hydnum, and the resultant growth has persistently failed to show any resemblance to Ozonium or Phymatotricrum.

In this connection, the writers have found a species of Coprinus also associated with roots previously infected by root rot. Sporophores of this fungus have appeared repeatedly on infected root material kept in moist chambers in the laboratory and on infected plants in the field. The possible genetic relation between this Coprinus and the Ozonium that causes root rot is yet to be determined.

SUMMARY

Phymatotrichum omnivorum, the cause of cotton root rot, has been proved to overwinter on infected live roots and as sclerotia in the soil. It may overwinter as spores but this has not been demonstrated.

Although the tops of cotton plants are killed by frost, the taproots, lateral roots, and rootlets may remain alive through the winter, and many survive until late the following summer. Root rot was present on roots found in field excavations during the winter and spring months. The viability of the fungus on overwintered roots was demonstrated by isolating the organism from the infected roots and by inoculation of sound turnips and cotton plants with overwintered, infected roots. *P. omnivorum* was viable on the living although infected roots but not on roots that were infected and decayed. This was in contrast with successful isolations of *F. vasinfectum* from cotton plants with wilt, even after the plants had been dried for a year.

In inoculations of cotton plants, infection was obtained with inoculum consisting of the taproots of plants which had succumbed to root rot only one day to two weeks earlier; while no infection resulted from inoculations with taproots from plants which had wilted from three to nine weeks pre-

viously. Infected roots were cut into sections 2 cm. long, which were placed in individual soil chambers. Strands from the sections which included living tissues but not from the sections of the same roots which included only decayed tissues. The disease not only remains viable on the living roots during the winter, but studies in two fields showed that root rot continued to spread during the winter, the maximum spread observed being twelve feet in one row. Root rot survives on the roots of many weeds as well as on cotton roots and may spread from these weed roots to succeeding crops. These results support the conclusion that *P. omnivorum* does not survive in an active condition on decayed roots but overwinters by continued though slow growth and spread on living, infected roots, and that overwintering on living roots is an important factor in the survival of root rot.

Excavations made in fields apparently free from overwintered roots but in which root rot was appearing on the new cotton plants revealed the presence of large numbers of sclerotia, which apparently were responsible for overwintering of the fungus in these fields. These observations confirm the work of King and Loomis and of Neal. Sclerotia have been produced also in soil chambers in the laboratory and in cultures on synthetic media. The sclerotia germinate readily and produce strands typical of *P. omnivorum,* and the same sclerotia may germinate at least five times under laboratory conditions. Cotton plants were successfully inoculated with a pure culture isolated from a sclerotium and with growth from sclerotia placed directly in the soil, and new sclerotia developed in these containers.

By dissection and microscopic study of Phymatotrichum spore mats from the field, it was possible to demonstrate actual continuity of the spore-bearing hyphae of the spore mats with the subterranean, typical Ozonium growth. Low percentages of germination have been obtained with Phymatotrichum spores, but no successful growth or infection from them has yet been secured. A Hydnum, frequently associated with plants killed by Phymatotricum root rot, does not resemble this fungus in pure culture, and definite connection of the Hydnum with the Phymatotrichum is yet to be determined. It is not yet known whether any spore stage is involved in the overwintering of the root-rot organism.

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FURTHER OBSERVATIONS AND EXPERIMENTS ON THE CURL DISEASE OF RASPBERRIES[1]

C. W. BENNETT

For more than thirty years the disease generally known as curl has been recognized as one of the most common troubles affecting raspberries and, in the case of two or three of the more susceptible varieties, has been one of the most destructive diseases of this plant. Within the raspberry group there is a wide range of varietal susceptibility. The disease is widely distributed on the variety Cuthbert and occurs more or less extensively on other red varieties, such as Latham, Viking, and Marlboro. The Cumberland and Gregg are the most susceptible of the more popular black varieties. On red and black varieties symptoms of curl are practically identical under Michigan conditions, and this similarity has been tentatively accepted in the past as proof that the disease on both types of raspberry is caused by the same virus. Experimental evidence to confirm this view has not been produced.

Very few cross-inoculation experiments between red and black varieties have been reported. Smith (9) inoculated four black-raspberry plants from a red-raspberry source but obtained negative results. He attributed this failure to secure infection to the large size and lack of succulency of the inoculated plants and stated that the virus from the two sources probably would be found identical. In a previous publication (1) the writer has reported negative results from inoculations in which transfer from red to black varieties was attempted.

A further study of this disease under field conditions has been made during the seasons of 1926, 1927, and 1928. An excellent opportunity for extensive observation has been offered through the cooperation of representatives of the Orchard and Nursery Inspection Service of the Michigan State Department of Agriculture who, in the course of their work, have made two inspections each season of an average of more than a thousand acres of raspberries. Through the courtesy of E. C. Mandenberg, in charge of this service, records of these inspections have been made available for study whenever desired. Fields in which severe infestations have occurred, therefore, have been readily located in this manner and the development of the disease on all of the common susceptible varieties has been studied in fields rogued for control as well as in many fields where no measures have been taken to hold the disease in check. The information

[1] Most of the studies reported in this paper were made while the writer was associated with the Department of Botany, Michigan State College.

gained from these observations indicated that in 1925 the occurrence of curl on black varieties was very rare in the chief raspberry sections of Michigan. Many of the well-informed growers considered black varieties to be immune. Even at that time, however, in the plantings in which curl was known to occur, it spread rapidly and was recognized as a serious disease. Since 1925 the number of infected plants of susceptible black varieties has greatly increased in nonrogued fields and the disease has spread over a much wider area, embracing a large section in the southwestern part of the State.

Plants affected by curl in 1928 were found in a higher percentage of the fields of the black variety, Cumberland, than had been noted prior to that time. In most plantings only a few plants were infected, indicating a relatively recent introduction of the disease. This spread of curl in black varieties has seemed to bear no relation to the occurrence of the disease in the red varieties grown in the immediate vicinity. It is not at all unusual to find curl-free plantings of black raspberries which have grown adjacent to badly infested red-raspberry patches for a number of years.

Apparently, these field observations on raspberry curl are best explained by assuming that there are two distinct causal viruses which produce similar or identical symptoms. In 1925 inoculation tests were started to throw additional light on this question. These have been repeated and expanded each season until the evidence now seems fairly conclusive that we are concerned with two distinct curl viruses that thus far have been separated chiefly on the basis of varietal range of plants susceptible to infection.

For the sake of clarity, the terms alpha and beta will be used in referring to the curl viruses from the two sources. By alpha-curl virus is meant the one common in Michigan on the Cuthbert variety of red raspberry; by beta, the curl virus found in that State on the Cumberland variety of black raspberry.

The varieties used for most of the inoculations here reported are Cuthbert, Cumberland, and the purple or hybrid variety, Columbian.

ALPHA VIRUS FROM RED RASPBERRY PLANTS, TRANSMISSIBLE TO RED BUT NOT TO BLACK VARIETIES

It has been demonstrated many times that the virus of curl (alpha virus), common to red raspberries in Michigan, is readily transmitted by means of the aphid, *Aphis rubiphila*. Records of controlled experiments are available in which 230 red-raspberry plants have been inoculated from plants affected by this virus. Infection has occurred in 137 of these plants. During this same period, 115 black-raspberry plants have been inoculated

in the greenhouse or in cage experiments with virus from the same source, but none of these plants have shown signs of curl.

In the spring of 1926 a one-year-old planting composed of three rows (90 plants) of the variety Cuthbert and five rows (150 plants) of the variety Cumberland, both known to be very susceptible to curl under field conditions, was selected for a field test of the alpha-curl virus. Numerous aphids were reared under cages on Cuthbert plants affected by curl. These aphids were used to inoculate all of the plants in the field, an estimated number of more than one hundred aphids being released on each plant. The inoculations were made June 21. By July 30, fifty-one of the Cuthbert plants were showing marked symptoms of curl. They were employed to rear more aphids which were placed on the Cumberland plants. Again, in the spring of 1927, aphids were placed on the black-raspberry plants, also subjected to infection from natural migration of aphids from the Cuthbert planting. No curl developed in the Cumberland plants by the middle of the summer when ten of these plants were inoculated with the beta virus. Three of the inoculated plants became diseased. In the early spring of 1928, eighty-four of the Cuthbert plants were affected by curl and, of the Cumberland plants, only the three plants infected by the beta virus showed curl symptoms.

In February, 1928, an experiment was conducted to test thoroughly the alpha virus under greenhouse conditions on a number of red, purple, and black varieties of raspberry and on Lucretia dewberry. Ten potted plants of each variety (Table 1) were selected when about three inches high and subjected to the feeding of more than a hundred aphids. Five days later the aphids were killed by means of a spray of nicotine sulphate. After the plants had recovered from the effects of the feeding of these many aphids, they were again inoculated. This was repeated until five large lots of aphids had been placed on each plant. This experiment was performed in a greenhouse where curl on the variety Cuthbert was the only virus disease known to be present. As shown in columns one and two of table 1, all the Cuthbert plants became infected, while none of those of the black varieties showed signs of disease.

It may be noted that one of the plants of the red variety, King, became infected. This variety is very resistant to infection, although, after plants become infected, they show very marked symptoms of curl. During the five years in which curl has been under observation in Michigan only two affected King plants have been found, despite the fact that plantings of this variety are abundant in locations where they are subjected to chances of infection throughout the growing season. One of the Lucretia dewberry plants became infected also and leaf curling and stunting were severe.

Under field conditions no infected dewberry plants have been observed, and, so far as is known, this is the first recorded occurrence of curl on this type of cane fruit. Curl, however, does occur to a limited extent in fields of Eldorado blackberry.

BETA CURL VIRUS FROM BLACK RASPRERRY TRANSMISSIBLE TO RED VARIETY, CUTHBERT

Experiments in Michigan, extending over a period of four years, have shown that the type of curl peculiar to the black variety, Cumberland, is

TABLE 1.—*Results of multiple inoculation of varieties of raspberry and Lucretia dewberry with the alpha and beta viruses by means of large numbers of aphids, Aphis rubiphila*

Variety inoculated[a]	Alpha virus		Beta virus	
	No. of plants inoculated	No. of plants infected	No. of plants inoculated	No. of plants infected
Cuthbert (R)	10	10	10	4
King (R)	10	1	10	0
Columbian (H)	10	4	10	10
Plum Farmer (B)	10	0	10	0
Gregg (B)	10	0	10	6
Cumberland (B)	10	0	10	10
Lucretia Dewberry	10	1	10	0

[a] R = red variety; H = hybrid variety; B = black variety.

readily transmitted to healthy plants of this variety and also to Gregg. This virus also can be transferred to the red variety Cuthbert, now known to be susceptible to both types of curl virus.

To obtain additional information regarding the varietal range of the beta virus, an experiment similar to that outlined for red raspberry curl was made in which large numbers of aphids were placed on potted plants at five different periods of growth. Results of this experiment are presented in columns three and four of table 1. Of the black varieties, as indicated by this test, Plum Farmer is very resistant, if not immune. The variety Cuthbert, although susceptible, has consistently shown a lower percentage of infection than in those experiments in which the alpha virus was used.

After the Cuthbert variety was found to be susceptible to infection by the beta virus, transfers of this virus were made from infected Cuthbert plants back to Cumberland plants. The results of one of these series of experiments are given in table 2.

Here again, in the check plants of this experiment, it will be noted that no infection of black varieties occurred in inoculations in which the alpha virus was used. Infection of susceptible black varieties occurred in all cases in which the beta virus was used, regardless of whether the immediate source of inoculation was a red or a black variety.

TABLE 2.—*Results of cross inoculation of the Cuthbert and Cumberland varieties with alpha and beta viruses*

Varietal source of inoculum	Type of virus	Variety inoculated	No. of plants inoculated	No. of plants infected
Cuthbert	Alpha	Cuthbert	30	22
Cuthbert	Alpha	Cumberland	25	0
Cumberland	Beta	Cumberland	30	12
Cumberland	Beta	Cuthbert	30	8
Cuthbert	Beta	Cuthbert	30	13
Cuthbert	Beta	Cumberland	30	19

Cuthbert plants affected by the alpha virus were next inoculated with aphids from Cumberland plants affected by the beta virus. Two plants were used and large numbers of aphids applied. Transfers were later made from these plants to healthy Cuthbert and Cumberland plants. Other transfers were made as recorded in table 3.

In this series of inoculations, infection occurred on Cumberland plants in all tests in which transfers were made from Cuthbert plants which had been inoculated with the beta virus, regardless of whether the alpha virus was present. This test indicates that the Cuthbert plants affected by the alpha virus remain susceptible to infection by the beta virus and that the beta virus can be recovered from such plants. It seems that the alpha and beta viruses may thrive in the same plant for an indefinite period. This, however, cannot as yet be conclusively demonstrated experimentally, for, while the beta virus may be separated from a mixture of the two by making transfers to Cumberland plants, there is no means with the varieties used in these tests whereby the alpha virus may be separated from a like mix-

ture. However, a more complete study of the varietal ranges of susceptibility to these two viruses may demonstrate their separability by methods similar to those employed in the isolation of the virus of black-raspberry curl.

FURTHER EVIDENCE SUPPORTING THE VIEW THAT TWO DISTINCT VIRUSES ARE RESPONSIBLE FOR CURL IN RASPBERRIES

Regardless of the origin or possible initial identity of curl virus, it seems quite evident from the experimental work done that at present there are two viruses which differ with regard to certain symptoms manifested by inoculated plants. The origin of two such similar viruses from independent sources would seem less probable than a derivation of one from the other or both from a common source. If the latter hypothesis is

TABLE 3.—*Results of inoculations from plants having the alpha and beta viruses present separately and in combination*

Varietal source of inoculum	Type of virus	Variety inoculated	No. of plants inoculated	No. of plants infected
Cuthbert	Alpha	Cuthbert	10	7
Cuthbert	Alpha + Beta	Cuthbert	20	11
Cuthbert	Alpha + Beta	Cumberland	20	9
Cuthbert	Beta	Cumberland	20	12
Cuthbert	Alpha	Cumberland	20	0

accepted as the more probable, the justification for the assumption that the viruses are fundamentally different manifestly depends upon the stability of virus from red and black raspberry under different environmental conditions. The history of the introduction and spread of curl virus in Michigan, were it known, would probably shed light of fundamental importance on this problem. The rather complete separation of the two types of virus in the State probably is due to an early introduction of what is now the alpha virus on the variety Cuthbert and to the continued propagation of this virus for many years in sections where black varieties are rare or resistant to curls of all types. In recent years the introduction and extensive use of the curl-susceptible Cumberland have introduced a new factor into the curl problem. The first Cumberlands grown in the State probably were free from curl and remained so until the introduction of a virus capable of producing infection on such plants. The extreme scarcity of this

disease on black varieties, in 1922, and the rather rapid spread during the last five years, coupled with the fact that curl on wild black raspberry is extremely rare, indicate a relatively recent introduction. It has been generally accepted that curl has been more prevalent on black raspberries in certain parts of Ohio, Indiana, and Illinois, where black varieties are grown extensively, than in Michigan. With the interstate shipment of nursery stock the introduction of the beta virus from outside sources may have occurred a number of times. This may explain the occurrence, but rather limited distribution, of this virus in Michigan. It might be expected that the beta virus would be the predominant type on all susceptible varieties of raspberry in sections where red and black varieties have been grown for several years in the presence of the disease. It is probable even that the alpha virus would not maintain its identity under such conditions but would become mixed with the beta virus to such an extent as to be unrecoverable in a pure state by the known methods of separation.

The alpha virus has, in all probability, been present in Cuthbert plants in Michigan for more than fifty years. This long association with a single variety may have had a very decided influence in modifying properties of this virus. Carsner's (3) results in attenuation of the virus of sugar-beet curly top indicate that at least some viruses may be rather unstable and subject to modification under certain conditions of environment. The alpha virus may thus be merely a form of that peculiar to black varieties but changed sufficiently to influence its varietal range by its long association with the Cuthbert variety. All attempts, however, to modify either type of curl virus have failed to indicate that this may be easily accomplished. It seems convenient for the present to consider these as separate viruses, since a rather clearly defined distinction seems possible. This decision is reached, however, with the full recognition that further experimental results may render this position untenable.

The two curl viruses may be characterized briefly as follows:

Alpha curl virus. Produces severe symptoms of curl on the Cuthbert variety of red raspberry but does not affect black varieties. Causes very mild symptoms of curl on the hybrid variety, Columbian. Plants of this variety recover.

Beta curl virus. Produces severe symptoms of curl on the red variety Cuthbert, the black variety Cumberland, and the hybrid variety Columbian.

EFFECT OF BETA VIRUS MORE SEVERE THAN ALPHA ON THE HYBRID VARIETY COLUMBIAN

The Columbian is the most popular purple variety grown in Michigan and New York. It is believed to be a seedling of Cuthbert found growing

near a planting of the black variety, Gregg. This ancestry is significant since the Cuthbert is susceptible to both the alpha and beta viruses and the Gregg is susceptible to the beta virus only. Columbian plants affected by curl have not been observed in Michigan, although the variety has been under observation since 1922. It has been observed frequently in close proximity to Cuthbert plants affected by curl. Two instances were noted in which rows of Columbian plants grew within eight feet of and parallel to rows of badly curled Cuthberts for periods of three and five years, respectively, without contracting the disease. In July, 1928, however, a badly curled Columbian plant was found near Ravenna, Ohio. There were curled black-raspberry plants growing in the immediate vicinity and it is assumed that infection came from these plants.

Young Columbian plants have been inoculated with the alpha virus in seven series of tests. The plants have shown a mild form of leaf curl after twenty to thirty days. The margins of the leaflets curve downward and roll slightly inward. The plants are not stunted and the deeper green, characteristic of the disease in other varieties, is not, in most cases, evident. Under favorable conditions for growth, symptoms may soon disappear. In table 1 are recorded the results of one series of inoculations with the alpha and beta viruses in which large numbers of aphids were used and each plant was inoculated several different times. The plants from these tests were grown through the season of 1928, heeled-in in the fall, and planted in the greenhouse in February, 1929. The plants inoculated with the beta virus were very much stunted and showed severe symptoms typical of this disease on susceptible black plants. All of the plants inoculated with the alpha virus produced canes as stocky and vigorous as those produced by the healthy check plants. Mild symptoms of curl were evident on four of these plants. Figure 1 shows two plants of this series, one affected by the alpha virus and one by the beta virus.

The Columbian variety has been used as a source of inoculum in three experiments involving sixty plants, in an effort to transmit the alpha virus through a hybrid variety to Cumberland. All inoculations from Columbian and also those from the purple variety Haymaker have failed to produce disease in inoculated black plants.

RECOVERY OF COLUMBIAN PLANTS INFECTED WITH THE ALPHA VIRUS

In the case of the great majority of virus diseases plant pathologists are agreed that plants once affected rarely recover. Masking of symptoms, due to certain environmental conditions or to the nature of the plant involved, is, however, now known to be a rather common occurrence with a number of the mosaics. Complete recovery with loss of the virus is more rare. A few such cases have been reported.

Brierley (2) observed the production of apparently healthy shoots from a tomato plant that had been affected by mosaic but had lost most of its leaves. Inoculations from these shoots to healthy tomato plants failed to produce disease.

Verwoerd (11) has more recently reported a similar occurrence on tomato. Cuttings having mottled leaves were made from mosaic plants. In two instances cuttings developed into normal-appearing plants from which no infectious material could be obtained. It is not stated whether

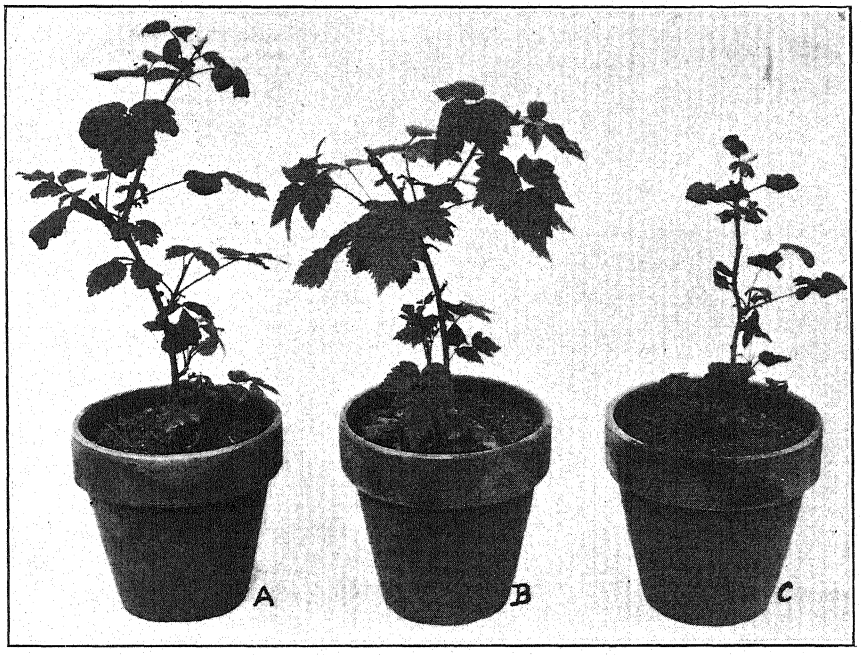


FIG. 1. Symptoms produced by the alpha- and beta-curl viruses on the purple or hybrid Columbian variety of raspberry. A, Columbian plant affected by the curl virus (alpha) from red raspberry. B, Healthy Columbian plant. C, Columbian plant affected by the curl virus (beta) from black raspberry.

these cuttings were tested when they were made to determine whether the virus was present at that time. It would have been interesting had this been done in view of the fact that Kraybill et al. (4) have shown that in one type of tomato mosaic there is present in diseased plants a separable fraction that will produce certain abnormal symptoms though apparently incapable of reproduction.

Recovery of certain varieties of sugar cane from mosaic has been found by Kunkel (5) to be a common occurrence. The tissues in the terminal

bud of the plants may recover and produce normal leaves after which the older, diseased leaves die and drop. In other instances the stools produce healthy shoots. Plants are subject to reinfection.

In transmission of streak from corn to sugar cane, Storey (10) found that the inoculated cane plants produced mild symptoms of disease and later recovered.

According to Severin (8), healthy branches occasionally are observed growing from plants of *Chenopodium leptophyllum* badly affected by sugar-beet curly top. Severin also found that inoculated plants of quail brush, *Atriplex lentiformis,* recovered from the effects of the curly-top virus. The plants after recovery were shown to be subject to reinfection.

In most varieties of raspberry there is no evidence of recovery from curl. Affected plants may live several years, gradually becoming more and more dwarfed until they die. In the case of the hybrid variety Columbian, however, there is evidence to indicate that plants regularly recover from the effects of the alpha virus after a period varying from a few months to two years and that the new growth, at least, is free from the virus. In the first series of inoculations with this variety made in 1924 very mild symptoms of curl were produced but the inoculated plants later seemed to recover. This apparent recovery was more extensively tested in 1925.

On February 20, 1925, ten Columbian tips growing in six-inch pots were inoculated with the alpha virus from badly diseased red-raspberry plants. Symptoms of curl developed on six of them. Transfers of aphids from each of these plants to five healthy Cuthberts produced curl in each lot of red raspberries inoculated. No curl virus was obtained from the four plants showing no symptoms. Five of the infected plants were transferred to the field in June and the sixth was transferred to a butter tub outside of the greenhouse. All of these plants showed curl symptoms throughout the summer of 1925. Transfers of aphids to healthy Cuthbert plants three different times during the summer showed the virus still present in the plant growing in the tub outside of the greenhouse. This plant showed mild symptoms throughout the season of 1926. In February, 1927, it was brought into the greenhouse where it made a vigorous and apparently normal growth. (Fig. 2.) Transfers of aphids from this plant to healthy Cuthberts no longer resulted in the production of curl. The plants in the field produced a very vigorous growth and no symptoms of curl were evident in 1926, the season following infection. Eight more Columbian plants were infected in February, 1926, and later were planted in the field. These showed symptoms of curl during that season but none were visible in 1927. Transfers of aphids in 1927, from these and from

the five infected plants placed in the field in 1925, produced no symptoms of curl on healthy Cuthbert plants.

The plant in the butter tub, which had recovered from infection with the alpha virus, was tipped and the tips as well as the mother plant re-

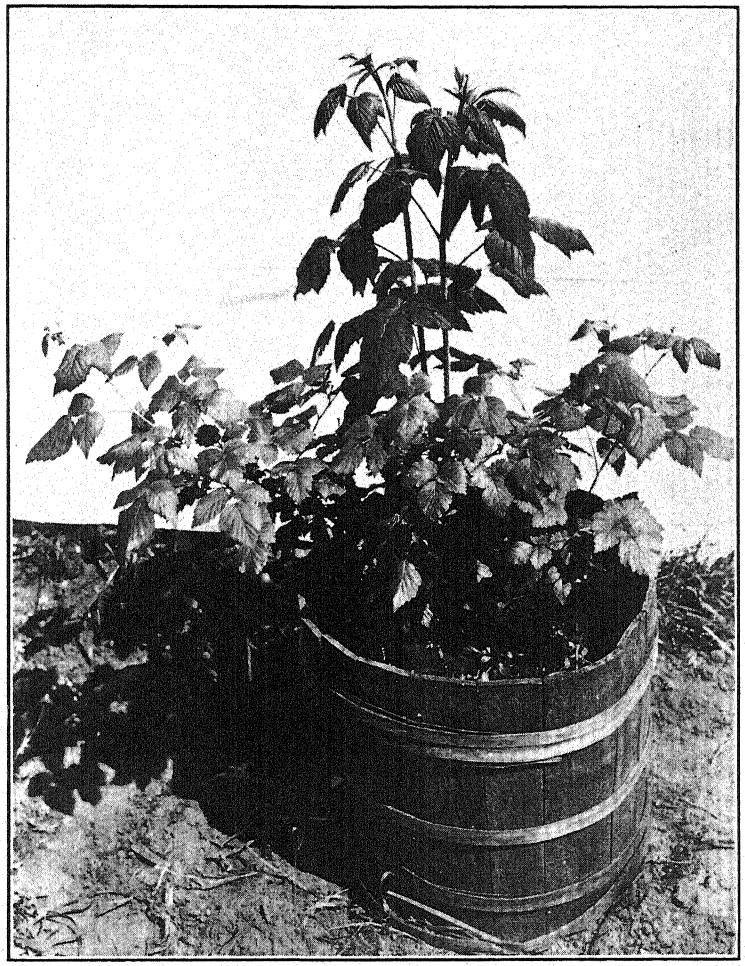


FIG. 2. A Columbian plant which was infected by curl virus from red raspberry February, 1925, and which recovered from the disease. Photographed April, 1928.

moved to Wooster, Ohio, in the fall of 1928. This plant and eight tips were planted in the greenhouse in February, 1929. Four tips and the mother plant were inoculated with the alpha virus. Infection was obtained in the mother plant and in three of the tips. The four remaining tips were in-

oculated with the beta virus and all developed marked symptoms of curl. Twenty tips from the plants inoculated in 1925 and 1926 and grown in the field also were selected and ten inoculated with the alpha virus and ten with the beta virus. Three and seven plants, respectively, became infected.

These plants, which had recovered from infection with the alpha virus, seemed no less susceptible to either the alpha or beta virus than plants which, so far as known, had had no contact with curl virus. The evidence available seems to indicate that the rate of growth influences speed of recovery, rapidly growing plants apparently recovering in a shorter time than those of less rapid growth. The recovery of rapidly growing Columbian plants from the influence of the alpha virus, together with the scarcity of the beta virus in regions where this variety is extensively grown, probably accounts for the fact that curl has been very rarely observed on plants of this variety.

FURTHER EXPERIMENTS IN THE TRANSMISSION OF CURL BY APHIDS

There are four species of aphids known to feed on the raspberry, namely, *Aphis rubiphila* Patch., *Amphorophora rubi* Kalt, *Amphorophora sensoriata* Mason, and *Amphorophora rubicola* Oestlund. The first of these was shown by Rankin and Hockey (7) to be a vector, and their results have been confirmed by a number of other investigations. Reports are meager regarding the other species. Smith (9) reported no infection in twenty plants inoculated with *Amphorophora rubi*. Bennett (1) also failed to obtain infection with this aphid in a limited number of inoculations. No one has reported inoculation tests with the two remaining species.

Since the species of Amphorophora consist of large and very active insects and since, if vectors of curl, they would be very important in dissemination, especially over long distances, it was thought worth while to conduct some rather extensive tests with all of those obtainable. *Amphorophora rubicola* has not been available in sufficient numbers for complete tests, although one series of inoculations has been made. In this experiment ten healthy Cuthbert plants were inoculated with five aphids, each. No disease developed on these plants, while, in the ten check plants on which *Aphis rubiphila* was used, five developed curl. The remaining two species have been used repeatedly and in large numbers. During the seasons of 1926, 1927, and 1928, 65 healthy plants have been inoculated with *Amphorophora rubi* and 35 with *Amphorophora sensoriata*. No curl developed on any of the plants inoculated. In March, 1928, ten healthy Cuthberts were selected and fifty specimens of *Amphorophora rubi* transferred from curl plants to each of the healthy plants. Five days after the

transfer these aphids were removed and fifty more were placed on each plant. Ten other plants were similarly treated, using *Amphorophora sensoriata,* and ten plants were inoculated with ten individuals of *Aphis rubiphila* per plant. Four of the ten plants inoculated with *Aphis rubiphila* became infected; none of those inoculated with *Amphorophora rubi* or *Amphorophora sensoriata* developed curl symptoms. These experiments were repeated in April, 1928, with essentially the same result.

Other experiments were conducted in which *Amphorophora rubi* and *Aphis rubiphila* were transferred from a Cuthbert plant having a combination of yellow mosaic, a medium type of mosaic, and curl. In one experiment *Amphorophora rubi* transmitted the two mosaics as a single virus to six of the ten plants inoculated, but no symptoms of curl developed. As a part of the same experiment, *Aphis rubiphila* was transferred from this plant to healthy Cuthberts with the result that four of the ten inoculated plants became affected by curl. None, however, showed any symptoms of mosaic. This experiment was repeated twice.

The beta curl virus was tested in a similar way on the Cumberland variety. A plant affected by curl and a medium type of mosaic was used from which to transfer the two species of aphids to healthy Cumberland plants. Here, again, in experiments in which *Aphis rubiphila* was used, curl was transmitted, and in transfers of *Amphorophora rubi* only mosaic was transmitted.

More experimental data should be available before any definite conclusions are drawn regarding the capabilities of *Amphorophora rubicola* for curl transmission. In the case of *Amphorophora rubi* and *Amphorophora sensoriata,* however, it seems reasonably certain that, if these species are capable of functioning as vectors of either of the curl viruses, they do so very rarely. It seems quite probable that neither of these species will transmit curl. Very little information regarding the underlying causes for the failure of these species to act as vectors is available. There is, of course, a possibility of a biological relationship involving *Aphis rubiphila* in the life cycle of a causal agent. However, if such is the case, experimental evidence indicates that the incubation period of the virus in the insect must be very short. Curl has not been transmitted artificially and indications are that the virus when outside of the plant is very sensitive to environmental conditions. It has been thought that this may possibly have a bearing on the apparent specificity of *Aphis rubiphila* in transmission in that this particular insect may be devoid of an inactivating substance which may conceivably be present in other species. This possibility as well as a study of tissues on which the different species feed would seem to offer fields for profitable investigation.

APHIS RUBIPHILA IN ALL STAGES OF DEVELOPMENT CAPABLE TRANSMITTING BOTH VIRUSES

In all of the earlier inoculation experiments with *Aphis rubiphila* the insects were used without regard to size of stage of development. After Rankin (6) reported transmission of mosaic on raspberries only with the younger instars of *Amphorophora rubi* and suggested that failure in his experiment to obtain transmission of mosaic by means of *Aphis rubiphila* may have been due to his using noninfective stages of the insect, it was determined to test the potentialities of different instars of *Aphis rubiphila* in the transmission of curl. It was found that aphids of this species molt so rapidly that it is difficult to keep accurate trace of instars where relatively large numbers of insects are used. For this reason plants were inoculated with aphids of different sizes, without regard to instars but representing progressively older insects from very young ones, 24 hours old or less, to and including wing forms, making the divisions small, medium, adult-wingless, and adult-wing. For these experiments, with the exception of the wing forms, colonies of first, second, third, and fourth generations from the stem-mothers were used. The aphids were reared on curl plants and removed from the inoculated plants after a period of twenty-four hours. Twenty aphids were placed on each plant with the exception of the plants inoculated with the wing forms, on which ten aphids per plant were placed. As shown in table 4, infection with both viruses was produced by aphids of all the sizes used.

TABLE 4.—*Results of an inoculation experiment with Aphis rubiphila in which insects of different sizes were used*

Size of aphid used	Cuthbert plants inoculated with the alpha virus		Cumberland plants inoculated with the beta virus	
	No. of plants inoculated	No. of plants infected	No. of plants inoculated	No. of plants infected
Small	10	2	10	3
Medium	10	4	10	3
Adult-wingless	10	5	10	6
Adult-wing	10	3	10	7

It has been demonstrated repeatedly that the wing form will transmit curl, and it seems quite evident that this form is very important in long-distance dissemination of this disease. During the seasons of 1927 and 1928 a number of instances of current or previous-season infection of

single plants were noted in fields which were being systematically rogued. In several instances the nearest known curl plant was not closer than a quarter of a mile and in two instances only one curl plant was discovered within a radius of a half mile. In sections where either of the curl viruses is present it should be expected that an occasional curl plant will be found even in fields which are carefully rouged. Such invasions of plantings from outside sources are very easily checked if affected plants are destroyed promptly.

SUMMARY

The curl virus, which has a general distribution in the Cuthbert variety of red raspberry in Michigan, apparently will not produce infection in the curl-susceptible Cumberland variety of black raspberries. The virus of curl which occurs on the Cumberland variety is transmissible to the variety Cuthbert and can be transferred from this variety back to black raspberry. It is assumed that two viruses are involved. These have been designated, respectively, as alpha-curl virus and beta-curl virus.

The purple or hybrid variety Columbian shows marked symptoms of curl when attacked by the beta virus and mild symptoms when infected by the alpha virus. Plants attacked by the alpha virus remain susceptible to infection by the beta virus.

Columbian plants infected by the alpha virus completely recover after a period varying from a few months to two years and new growth is free from the virus. Plants that recover remain subject to infection by both the alpha and beta viruses.

Transmission of the two curl viruses was obtained only by means of *Aphis rubiphila*. No curl infection was obtained in experiments in which *Amphorophora rubicola, Amphorophora rubi,* or *Amphorophora sensoriata* was used. All stages of *Aphis rubiphila* including the wing form transmitted curl. The wing form is considered to be very important in long-distance dissemination.

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SOIL-REACTION EFFECTS ON PHYMATOTRICHUM ROOT ROT[1]

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INTRODUCTION

It has long been noticed that cotton root rot, caused by *Phymatotrichum omnivorum* (Shear) Duggar, is more serious in the black land regions of Texas, where the soils are alkaline in reaction, than in east Texas where many of the soils are slightly acid. A survey of fields in sixteen counties of Texas furnished preliminary evidence of a correlation between soil reaction and the occurrence and severity of root rot. The disease was found[3] in 34 per cent of fields with soils of pH 5.5 to 6.4, in 60 per cent of fields with soils of pH 6.5 to 7.4, and in 71 per cent of fields with soils of pH 7.5 and above. Furthermore, where root rot was found in acid soils, pH 5.5 to 6.5, damage from the disease was negligible as compared to the losses, ranging from 20 to 100 per cent, in the neutral or alkaline soils.

These results tended to show that root rot is destructive in neutral or alkaline soils but unimportant in acid soils. Experimental studies under controlled conditions were necessary, however, first, to prove that the correlation observed was actually with the soil reaction rather than with regional, climatic, or other factors, and, second, to find the critical soil reaction necessary to inhibit infection, to prevent spread, or to prevent overwintering of the fungus. The critical reaction may or may not be different for these various processes, and interruption of any one of these portions of the life history might serve to control root rot. The present paper includes summaries of some experiments which bear on this problem. Further work is in progress and will be reported later.

PRELIMINARY SOIL REACTION SERIES IN DRAIN TILES

In 1927 a series of 120 drain tiles, each 2 feet deep and 18 inches in diameter, were installed as containers for a preliminary experiment. The tiles were sunk in the ground, upright, nearly to the ground level, and filled with soil. Various materials were worked into the surface soil to change the reaction. Soil samples were taken periodically and the hydro-

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[2] The writers are indebted to Dr. G. S. Fraps for advice in the planning of experiments and to Mr. W. T. Carter for identification of the soils studied.

[3] Taubenhaus, J. J., Walter N. Ezekiel, and D. T. Killough. Relation of cotton root rot and Fusarium wilt to the acidity and alkalinity of the soil. Texas Agr. Exp. Sta. Bul. 389. 1928.

gen-ion concentration determined colorimetrically. It was found that the soil reaction changed to depths of 6 to 8 inches only, the reactions obtained in this surface soil ranging from pH 2.3 to 8.0, while the deeper soil remained nearly neutral or slightly alkaline. For instance, samples taken in one container in May, 1928, ran pH 4.3, 4.3, and 4.4, respectively, for the upper three inches; 6.1, 6.5, 6.9 for the next three inches; and 6.9, 7.1, 7.1 for six to nine inches deep.

Cotton was planted in each container in 1927, 1928, and 1929. The plants were thinned to approximately an even stand and inoculated each year with root rot in every container. The method used was that previously described[4] in which roots of naturally and recently infected plants are inserted next to the roots of the plants to be inoculated. Root rot was introduced successfully, by this artificial inoculation, into every container irrespective of the reaction of the surface soil. There were differences in the numbers of plants attacked by root rot and differences in the numbers that died, in the various series. Summarizing all the results without regard to the treatment used to adjust the reaction of the soil, the results given in table 1 were obtained for 1928. It will be noted that there were higher percentages of infection of plants in the neutral and alkaline soils than in the more acid soils and that twice as high a percentage of the plants was killed in alkaline surface soils as in acid soils.

It is of particular significance that the disease overwintered in some of the containers in each series in the winter of 1927 and also of 1928, appearing on plants of the succeeding crop prior to the inoculation. Acidification of the surface soil alone then appeared insufficient to prevent the survival of root rot on roots in neutral soil below the acid region. At the same time, the high acidity obtained in the surface soil in many containers was enough to cause injury to plants and to prevent growth of cotton plants in the six containers of the most acid series in 1928 and 1929.

INOCULATION EXPERIMENTS WITH ALABAMA SOILS

Surface soil material of Houston black clay and Oktibbeha clay was secured in 1928 from Alabama through the courtesy of Mr. J. F. Stroud. Four cans were filled with each soil, cotton was planted, and the plants in each can were later inoculated with root rot. As shown in table 2, root rot was able to attack these plants growing in soils from a region in which Phymatotrichum root rot is unknown. The amount of infection and the percentage of loss from root rot were much higher in the more alkaline soil than in the only slightly acid Oktibbeha clay.

[4] Taubenhaus, J. J., B. F. Dana, W. N. Ezekiel, W. J. Bach, and J. P. Lusk. A method of inoculation for Phymatotrichum root rot investigations. Phytopath. 19: 167–170. 1929.

TABLE 1.—*Results of preliminary series in drain tiles for 1928 season, considering containers with similar soil reaction together; the pH values apply to surface soil only, the subsurface remaining unaffected by the treatment.*

Average pH of surface soil, 0–8 inches	Number of plants included	Percentage of plants infected with root rot	Percentage of plants killed by root rot
2.9–3.1	0[a]	.....	.....
4.5–4.7	63	62	19
5.0–5.4	170	70	27
6.5–6.9	90	83	35
7.0–8.0	240	80	43

[a] On account of the excessive acidity in one series of six tiles, no stand was secured in 1928 or 1929.

INOCULATION EXPERIMENT WITH FIVE SOIL TYPES IN SMALL CONTAINERS

In the spring of 1928, an extensive experiment was set up primarily for the study of the incubation period and the development of root rot on the roots of cotton plants grown in five different soil types and inoculated at a definite time. Containers measuring two feet in each dimension were built with wood frames and with sides of wire screening and heavy tar paper. Eight containers were used for surface soil material of each of the five soil types listed in table 3. The containers were arranged side by side on the surface of the ground, and the spaces between and around them were filled in with soil to aid in maintaining natural temperature conditions. Cotton was planted, thinned to five plants; and, on August 14, two of the plants in each container were inoculated with two roots each from recently wilted cotton plants. One container of each soil type was opened at weekly intervals thereafter, the tops of the plants were secured by strings, and the soil was removed by washing (Fig. 1, c). The final series was held

TABLE 2.—*Results of inoculations of cotton plants growing in soil from Alabama; 30 plants per soil type*

Soil type	pH	Percentage of plants infected with root rot	Percentage of plants killed by root rot
Houston black clay	7.6	95	90
Oktibbeha clay	6.7	35	15

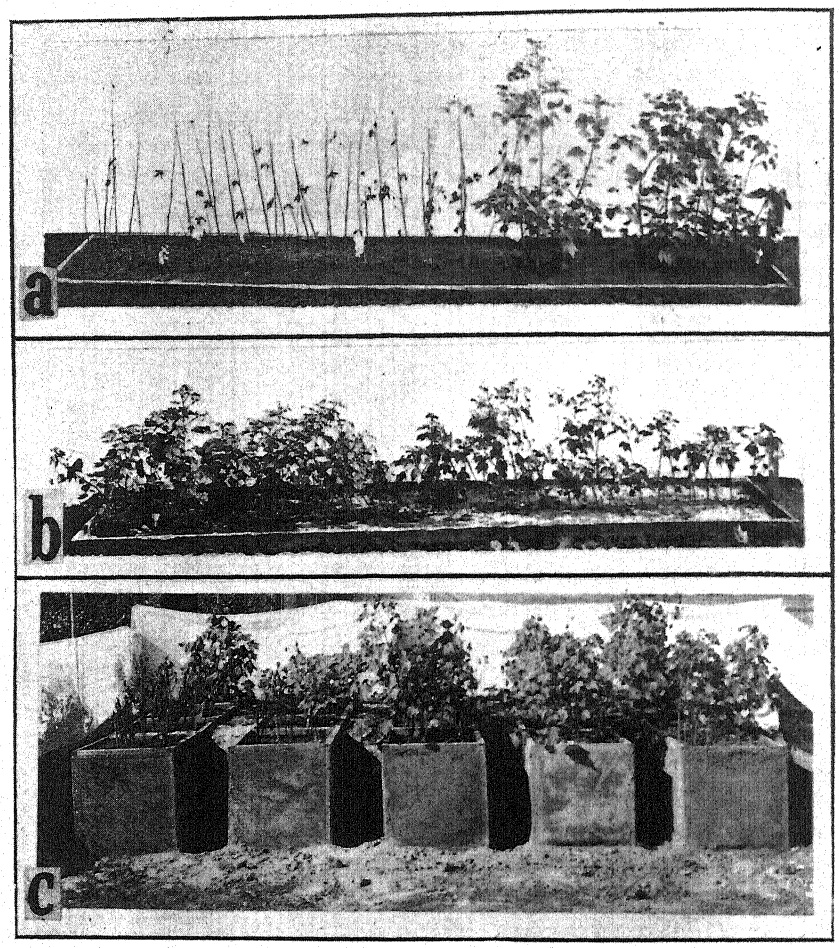


FIG. 1, a and b: Results obtained by November 15, 1928, from repeated inoculation at the left ends of the rows of cotton in boxes; a, of Houston black clay, pH 7.7, root rot spread more than halfway down the row; and b, of Tabor fine sandy loam, pH 5.8, no root rot.

c: Small containers of five soils used in experiment (Table 3) in which plants were periodically washed out. Photograph Sept. 18, 1928, showing surrounding soil already removed from around the five containers to be opened for final examination at that time.

until the spring of 1929, when it was opened and notes taken on the condition of the roots at that time.

The results for the first weeks were similar in the various soils. At the end of one week, there was no evidence aboveground of any injury to the inoculated plants. As the plants were washed out of the soil, the typical whitish to yellowish mycelial growth of the fungus was noted on

the roots of the inoculated plants along the portions of the roots adjoining the inoculum. After two weeks, there was still no evidence of the disease aboveground except for occasional plants which were freshly wilted, but

TABLE 3.—*Results of inoculation of 221 cotton plants grown in 8 containers per soil type and washed out at intervals after inoculation*

Soil type	pH	Average interval between inoculation and wilting of inoculated plants, days[a]	Percentage of plants with root rot, for entire experiment	Spread: Percentage of uninoculated plants with root rot found in washing out plants from fourth to seventh weeks after inoculation
Lufkin fine sandy loam	6.1	21.1	52	38
Crockett clay loam	6.2	19.5	58	67
Kirvin clay	6.4	19	68	81
Norfolk fine sand	6.8	15.6	63[b]	54[b]
Wilson clay	7.4	17	70	83

[a] Intervals calculated only from plants that wilted before time of washing out from the soil, and averaged for 10 plants each from series with Norfolk and Wilson soils and 11 plants from each of the other series.

[b] Figures include plants washed out after five weeks from the only container in the experiment in which the inoculation was apparently unsuccessful.

the mycelial growth on roots of the inoculated plants had extended over a more considerable portion of the taproots, and definite lesions were found on the portions of the roots first attacked. In three weeks, many inoculated plants had succumbed to the disease. Nine of the ten inoculated plants had wilted, and their roots were badly diseased, often to 30 or 40 cm. below ground. Lesions were beginning to be visible on the roots of plants adjacent to the inoculated plants.

By the fourth week, there were differences between the results in the different soil types. In the acid, Lufkin soil only the inoculated plants had become infected, while all five of the plants in the alkaline, Wilson clay were infected. Differences in spread were found also in subsequent examinations. As shown also by percentages in table 3, of the uninoculated plants examined from the fourth to seventh week after inoculation, ten of the sixteen plants from the Lufkin soil had escaped infection, while only two of the twelve uninoculated plants from the Wilson containers had escaped infection.

There were rather definite differences between the soil types with regard to the time elapsing between inoculation and the time when inoculated plants succumbed to root rot. In all types, some plants were observed to wilt by the twelfth, thirteenth, or fourteenth day after inoculation. The majority of inoculated plants had wilted by the end of the third week in all series. However, wilting of some of the plants in the acid soils was delayed longer than in the other soils, so that the average number of days between inoculation and wilting was greater for plants in the acid soils.

The final series of boxes was held until April, 1929. By this time, thirty-three weeks after inoculation, the roots of all plants in all five soils had become infected with root rot; and all were dead except for portions of roots in the Lufkin soil.

The Lufkin series was of further interest in that 6 of the 24 plants in the fourth to seventh harvests were affected by dark, gall-like excrescences along the taproots. These calluses resembled the effects of acid injury to cotton roots and perhaps resulted here from callusing following injury during cultivation. The calluses appeared of little importance in the growth of the plants.

This experiment showed that recognizable symptoms of root rot can appear on cotton roots in the five soil types used within a week after inoculation, while plants may wilt as a result of root-rot infection in 12 to 14 days in all the soil types, although, for the entire experiment, the average interval before wilting was longer in the more acid soils than in the more alkaline soils. Spread from inoculated plants to other plants in the containers was less in the more acid soil than in the other soils during the first seven weeks following infection, although by the following spring spread had occurred in all the soils. The most acid soil used here, pH 6.1, was not acid enough to prevent infection of inoculated plants and slow spread to adjacent plants.

INOCULATION EXPERIMENT WITH SEVEN SOILS, IN LONG BOXES[5]

In the experiments summarized above, the containers used were too small for detailed observation of the effect of soil differences on spread as distinct from initial infection. As has been reported elsewhere[6] an experiment was set up early in 1928 in which this difficulty was obviated by the use of larger, wooden containers 12 feet long, 3 feet deep, and 2 feet wide. Soils of seven different types were used, each in a single container. The soil material was dug out to a depth of three feet with each six-inch layer sacked separately and replaced, with each soil layer in its original depth, in the boxes at College Station.

[5] This experiment was installed by Dr. L. J. Pessin, formerly of this Division.

[6] Taubenhaus, J. J., and Walter N. Ezekiel. Recent studies on Phymatotrichum root-rot. Amer. Jour. Bot. 17: 554–571. 1930.

Cotton was planted in a row along the middle of each box in 1928 and again in 1929. During 1928, the plants at one end of each box were inoculated on June 22, August 8, and September 8. The results (Table 4) were in accord with the reaction of the various soils. Spread of root rot from the inoculated plants was successively greater in the soils with the higher pH values. No spread occurred in the most acid soils. Only one of the inoculated plants succumbed in the soil with pH 5.5 and not one plant in the Tabor soil with pH 5.8. (Fig. 1, a, b).

At the end of the growing season of 1928, the tops of the plants were cut off, but their roots were left undisturbed in the soil, and, on October 9, 1928, the roots of fifteen freshly infected cotton plants secured from nearby fields were inserted in each row to furnish additional material for possible overwintering. In 1929, cotton was planted again along the middle of each box without removing the roots of the 1928 crop. There was no inoculation in 1929.

TABLE 4.—*Summary of inoculation experiment with rows of cotton in long containers; inoculated repeatedly at one end of rows during 1928; no inoculation in 1929*

Soil type	pH of soil (0–2 feet deep)	Spread of root rot along rows during 1928, feet	Percentage of plants infected	
			1928	1929
Susquehanna fine sandy loam, shallow phase	5.5	0	2	8
Tabor fine sandy loam, shallow phase	5.8	0	0	0
Ochlockonee clay loam	6.3	4	27	59
Tabor fine sandy loam	6.7	4	29	34
Kirvin fine sandy loam	7.1	5½	30	80
Caddo fine sandy loam	7.6	7½	53	82
Houston black clay	7.7	7½	74	100

As shown in table 4, the disease overwintered successfully in every soil in which there had been any infection the preceding year, and in each soil it attacked a higher percentage of plants during the season of 1929 than in the previous year. The plants in the shallow phase of Tabor fine sandy loam were still free of root rot, while every plant in the Houston black clay was affected by September 12. Since the additional inoculum was inserted in 1928, it is possible that the increase of root rot in 1929 in some of the acid soils was due to this additional inoculum and that under field conditions root rot might instead have decreased.

While most of the soils used in this experiment were of approximately the same reaction to the depth used in the containers, the Tabor fine

sandy loam, shallow phase, in which no infection occurred either year, was distinctly acid only in the upper two feet and had a pH of 6.6 to 7.0 in the lower foot. This practically neutral subsoil apparently did not affect the results.

In the present experiment, the incidence and spread of root rot were found to correlate directly with the pH of soils held under controlled conditions. Root rot overwintered in all soils in which the disease had been able to infect cotton plants.

INOCULATION EXPERIMENT WITH DIFFERENCES IN DEPTHS OF ACIDIFICATION

In the spring of 1929, an experiment was started in which a single soil type, Lufkin fine sandy loam, was used in a series of 16 wooden boxes, 2 feet in each dimension. This soil was used in two ways, as indicated in table 5, either with 1 per cent of ground limestone added to the soil (on a soil dry-weight basis) or with the soil adjusted with sulphuric acid to pH 5.0. The amount of acid to use was determined with aliquot samples

TABLE 5.—*Inoculation experiment with cotton plants, six plants per wood box of Lufkin fine sandy loam; soil made alkaline by addition of 1 per cent ground limestone or acid by addition of sulphuric acid. Heavy inoculation, July 26, 1929. These results are of aboveground effect only, to end of season.*

Soil	Location of inoculum	Average interval between inoculation and wilting of inoculated plants, days	Number of plants succumbing to root rot
All alkaline (pH 7.2–7.3)	In upper foot of soil	20	6
	"	31	4
	In lower foot of soil	.....	0
	"	48	6
All acid (pH 4.9–5.3)	In upper foot of soil	.....	0
	"	.....	0
	In lower foot of soil	.....	0
	"	.....	0
Upper foot acid, lower foot alkaline	In upper foot of soil	78	1
	"	.....	1
	In lower foot of soil	.....	0
	"	.....	0
Upper foot alkaline, lower foot acid	In upper foot of soil	12	5
	"	21.5	6
	In lower foot of soil	.....	0
	"	.....	0

from the soil layers by the methods worked out by Fraps and Carlyle[7] for determining amounts of sulphur needed to adjust soils to desired acidities. In four containers, the entire two feet of soil was made alkaline, and, in another four, the entire depth was made acid; while four boxes were used with the top foot of soil acid and the bottom alkaline and four with the top alkaline and the bottom acid.

Cotton was planted June 11, 1929, and thinned July 26 to six plants per box, arranged in two short parallel rows. On July 26, the two plants at the same end of each row of plants were inoculated heavily with root rot. The inoculum consisted of the roots of freshly wilted cotton plants, trimmed down to exactly eight inches in length. Four roots were used for each plant inoculated. In two of each series of boxes, the inoculum was placed in the upper foot of soil by inserting it in holes bored to a depth of 10 inches with a soil augur; while in the other two boxes, it was placed in the lower foot of the soil in holes bored to 22 inches.

The results now available with this experiment are of the effects of root rot aboveground, since the roots have been left undisturbed. As shown in table 5, root rot killed most of the plants in the alkaline soil but none of those in the acid soil. Inoculation in the upper foot of the soil was markedly more sucessful than in the lower foot, irrespective of the reaction of these two soil layers. In the series in which the upper and lower layers were of different reaction, root rot appeared only where the inoculum was in the upper foot and in these boxes killed most of the plants in alkaline surface soil but only two of those in the acid surface soil.

The reaction of the acid soil selected on the basis of previous results appears to have been sufficiently acid to prevent inoculation with root rot under the conditions of this experiment. It did not interfere noticeably with the development of the cotton plants.

PRELIMINARY EXPERIMENT WITH SULPHUR FOR SOIL ACIDIFICATION[8]

The work referred to above indicated that root rot was more destructive and spread more in alkaline or neutral than in acid soils, whether this acidity was natural or obtained by acidification of the soil, and suggested the possibility of soil acidification for the control of root rot. The most economical and convenient method now available for acidifying soils in the field is to apply sulphur, which is slowly oxidized to sulphuric acid. This oxidation occurs more rapidly when the soil flora as well as other conditions in the soil favor oxidation. The present experiment was de-

[7] Fraps, G. S., and E. C. Carlyle. The basicity of Texas soils. Texas Agr. Exp. Sta. Bul. 400. 1929.

[8] This experiment was set up by W. F. Vogel, formerly Sulphur Research Fellow in this Division.

signed as a preliminary test of the possibility of reducing loss from root rot by the application of sulphur, and of the efficiency of eight different lots of commercial sulphurs, some of which lots included oxidizing materials. The results are combined below without regard to the various sulphurs used.

Surface soil material of Crockett clay loam was used in small metal containers some 10 inches and others 12¾ inches in diameter. There were four containers for each of the three rates of application of each of the eight sulphur materials. These materials were applied after the containers were filled, and were incorporated to a certain extent in the surface one or two inches of soil. The sulphurs were added June 12, 1928, and were still present in large lumps just below the surface as well as on the surface at the end of the season. It was apparent that, just as in the preliminary experiment in drain tiles (Table 1), the acidity resulting here was highly localized. This explained why the lower roots of some plants rotted from root rot, while parts of the taproots, closer to the surface, showed definite evidence of acid injury.

Cotton was planted and thinned to about four plants per container, giving a total of 14 to 16 plants for each application of each material and of 45 plants for the checks. Plants in all containers were inoculated with root rot by the usual method. Wilting of diseased plants was observed during the season, and at the end of the season all plants were pulled up for examination of their roots. It was found at this time that many of the dead plants in the containers had died apparently from acid injury, as evidenced by the characteristic enlarged cracked regions and the absence of Phymatotrichum strands or other symptoms of root rot. Plants with acid injury were more abundant in the series with higher rates of application of sulphur than in those with 5,000 lbs. per acre and were more abundant in the series in which the soil actually became highly acid than in the series of less acid soil.

Root rot, on the contrary, was less destructive in the more acid soils. Forty per cent of the check plants were killed by root rot, while only three per cent of the plants were killed in the series receiving 5,000 lbs. per acre, one per cent in the 10,000-lb. series, and none in the 15,000-lb. series.

The entire series of containers was held over winter and replanted to cotton in 1929. Despite repeated plantings, no stand could be obtained on account of the high acidity which had developed by that time.

In this preliminary experiment, excessive quantities of sulphur, confined almost entirely to the surface, reduced loss from root rot to a small fraction of the loss in the checks. There was, however, considerable acid injury to the plants, particularly in the most acid series. On account of

the uneven distribution of acidity, plants with the upper portion of the taproots injured by acid occasionally had the lower roots, extending into non-acidified soil below, destroyed by root rot.

FIELD TESTS OF SULPHUR FOR THE CONTROL OF ROOT ROT

A long-continued experiment with sulphur for the control of root rot was carried out at the former site of Substation No. 5, Bell County, with soil of a highly calcareous Bell clay. From 1920 until 1926, sulphur was applied yearly to various plats, in some cases at the rate of 10,000 pounds per acre. This did not exhaust the alkaline reserve even of the surface soil nor reduce the loss from root rot to a significant extent. Laboratory experiments by Fraps and Carlyle[7] have indicated similarly that it is impracticable to acidify highly calcareous soils by the application of sulphur.

Beginning in the fall of 1927, field tests[9] were carried on in seven localities in the State in soil types which are not highly calcareous and which accordingly might be acidified with quantities of sulphur that might be used in farm practice. These tests were initiated even before completion

TABLE 6.—*Inoculation experiment with cotton plants grown in small containers; results in the eight series with different sulphur materials combined here*

Sulphur added to soil	pH	No. of plants	Root rot		Sulphur injury	
			Percentage of plants affected	Percentage killed	Percentage of plants affected	Percentage killed
15,000 lbs. per acre	2.1–4.0	118	5	0	48	15
10,000 lbs. per acre	2.2–3.1	117	5	1	32	3
5,000 lbs. per acre	2.3–5.7	119	17	3	20	3
None (checks)	5.2–6.0	45	62	40	0	0

of the study of the field-survey results[3] which first demonstrated that the destructiveness of root rot in the field is correlated with the reaction of the soil. Empirical applications with various amounts of sulphur were made on the surface of the ground and incorporated somewhat into the surface soil. The tests showed that application of sulphur may acidify soils that are not highly calcareous; that excessive quantities of sulphur may cause acid injury of plants and may prevent obtaining a stand when the acidity

[9] These field tests were carried on by H. E. Rea, in cooperation with this Division.

TABLE 7.—*Results of sulphur experiment, data the same as in table 6, but arranged here by the average pH of the various series*

pH	No. of plants	Root rot		Sulphur injury	
		Percentage of plants affected	Percentage killed	Percentage of plants affected	Percentage killed
2.1–2.5	41	0	0	75	17
2.6–3.0	163	4	0	31	8
3.1–3.5	73	6	3	12	3
3.6–4.0	62	35	2	40	2
4.6–5.0	15	0	0	0	0
5.2–6.0 ..... (Checks)	45	62	40	0	0

becomes more intense than about pH 3.5; and that even so intense acidities as this will not control root rot if the subsurface remains alkaline and the disease survives there.

SUMMARY AND CONCLUSIONS

In a number of experiments, cotton plants were grown in containers filled with soils varying naturally in reaction and in other containers filled with soils in which the reaction was adjusted by the addition of various materials. Plants were inoculated artificially with root rot caused by *Phymatotrichum omnivorum*. The percentage of infection and the percentage of plants killed by root rot were higher in soils with the higher pH. Fewer plants were attacked in acid soils; the average interval between inoculation and wilting of plants was slightly longer in the acid soils; and root rot spread to a shorter distance as well as more slowly in acid soils than in neutral or alkaline soils. There was marked diminution of root rot at about pH 6.0 and no root rot with the soil at pH 5.0 to the bottom of the containers.

When only the surface layer of soil was acidified, without affecting the lower layers, the general correlation held true but was less pronounced. Root rot was reduced but still occurred in containers in which the surface was very acid while the subsoil was slightly alkaline. The disease also overwintered successfully in such containers.

Preliminary tests with sulphur for the control of root rot have been carried out in containers and in field plats. In both series, the sulphur was mixed only with the surface soil and acidified only the surface layer.

Root rot was reduced but not eliminated even with surface soil as acid as pH 3.4 when this acidity extended only to depths of three to six inches. Acid injury occurred in surface soils acidified to pH 2 to 4 even though the subsurface was still neutral or alkaline.

These results indicate the possibility of the use of sulphur for the acidification of soils as a method of attack against root rot but bring out some serious difficulties. Studies to determine the practical effectiveness of this method and to devise means of utilizing it under field conditions are now in progress and will be reported later.



STUDIES ON THE MOVEMENT OF THE CROWN-GALL ORGANISM WITHIN THE STEMS OF TOMATO PLANTS[1]

SPAS S. IVANOFF AND A. J. RIKER

INTRODUCTION

The movement which enables bacterial plant pathogenes to migrate from the point of entry into the surrounding tissue is of fundamental importance in relation to their pathogenesis. Once established within the host it appears that the further progress of the parasitic bacteria causing disease is influenced by the various factors which favor this movement. Since the movement of the bacteria through the tissue is important in relation to the development of the disease it seemed desirable to study the mechanism by which this was accomplished in connection with investigations on the crown-gall organism, *Phytomonas tumefaciens* (Smith and Town.) Com. S. A. B. The present paper gives an account of such studies on the movement of the crown-gall organism and certain other substances within the stems of tomato plants, primarily within a few hours after introduction of the bacteria through needle punctures.

Several workers of the last decade who have considered the crown-gall organism to be an intercellular parasite have dealt with this problem. Robinson and Walkden (7) report, "Both in *Chrysanthemum frutescens* and in *Nicotiana affinis* we have definitely demonstrated, by staining, zoogloeal strands of *B. tumefaciens* intruding along intercellular spaces and protoxylem vessels forming centers for pathological disturbance and gall-production along the tract." Riker (5) observed that, immediately following the puncture, a darkened area appeared, which tended to be parallel to the long axis of the plant and which was caused by the occupation of the intercellular spaces by liquid. He reports (6), "It seems unquestionable that the bacteria once inside such a wound would migrate to the limits of the avenue provided by the flooded intercellular spaces." Also he says (5), "The forces which govern this entry of the organism into the tissue are not definitely understood. The bacteria might conceivably be influenced by any or all of such factors as the collapse of drying tissue, negative pressure, sap rise, and motility with or without a chemotactic

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

These studies are a part of the work on the crown-gall project which is being carried on in cooperation with the United States Department of Agriculture.

This work has been supported in part by a grant from the special research fund of the University of Wisconsin.

stimulus." Hill (3), following the method of investigation similar to that employed by Nixon (4), by Beach (1), and later by Haber (2), reaches these conclusions: "It is evident from my observations of the early stages of the migration resulting from inoculation that, after a few hours following their introduction into the tissues, the bacteria can be recognized only as widely scattered clumps clinging in the intercellular spaces. This is due to the fact that the rate of growth is very rapid and that after a few hours the tips of the zoogloeae pass through the tissues leaving occasionally a few scattered clumps of bacteria. . . . The rate of migration of *B. tumefaciens,* or actually the rate of growth of the zoogloeae of this species, is so rapid that very early stages can be observed only in material killed within one hour after the bacteria have been introduced into the tissues. . . . The rate of growth of the zoogloeae through the intercellular spaces is from 0.029 mm. to 0.04 mm. a minute, the more rapid rate occurring in the early stages of the migration following the introduction of the bacteria into the young tomato stems. The rate is most rapid for the first 30 minutes following inoculation. After three hours the rate is reduced one half."

The difficulty of demonstrating the crown-gall organism within the host tissue following its introduction through punctures has been an obstacle to all the investigators of this question. The characteristic staining properties of this organism are so nearly those of the host tissue that an individual organism or even a small group of several hundred organisms might very easily be overlooked. To overcome this difficulty a method was sought which would render the location and identity of the crown-gall organisms more easily discernible.

MATERIALS AND METHODS

The culture of *Ph. tumefaciens* used in this investigation was the progeny of a culture originally isolated from black raspberry and purified, after several platings, by single-cell isolation. The pathogenicity of this culture was repeatedly demonstrated on tomato, tobacco, and apple.

Readily discernible substances were sought which could be employed in conjunction with a suspension of the crown-gall bacteria at the time of inoculation to direct attention to the presence of the pathogene. It was hoped that such substances, when mixed with the living crown-gall bacteria, might be carried along with the bacterial mass. Then, by reason of their ready visibility, they might direct attention to the position of the crown-gall bacteria themselves. It was thought desirable that some of the substances should be living and some of them nonliving.

The substances employed included two living bacterial cultures and one inert substance. The cultures were *Ph. insidiosa* McCul. and *Ph. michiganensis* (Smith) Com. S. A. B., which are both Gram-positive and

both nonmotile. They were secured through the courtesy of F. R. Jones and H. L. Blood, respectively. Because of their characteristic reaction to Gram's stain, these organisms are very easily discernible within the tissue. A single rod of either species may be seen and identified as a bacterial organism inside the tissue with very considerable accuracy. The inert substance chosen was Burri's India ink. Different lots of these substances were employed alone or in different combinations as follows: (1) *Ph. tumefaciens,* (2) a mixture of *Ph. tumefaciens* and *Ph. insidiosa,* (3) a mixture of *Ph. tumefaciens* and *Ph. michiganensis,* and (4) a mixture of *Ph. tumefaciens* and Burri's India ink.

Control inoculations were made with the following materials: (1) Crown-gall bacteria, (2) a mixture of crown-gall bacteria and India ink, and (3) a mixture of crown-gall bacteria, India ink and *Ph. insidiosa.* In all cases galls developed at the end of the usual incubation period.

The movement of inert substances and a correlation of their reactions with those of the living bacteria under the conditions of puncture inoculation were considered. Such a study, it was thought, might facilitate the analysis of the factors responsible for the movement and the shape of the bacterial masses in the plant tissues. The materials chosen for this purpose were Burri's India ink and crown-gall bacteria which had been killed by heating to 75°–80° C. for 45 minutes. After heating the bacteria were plated on bile agar, but no growth appeared after an incubation period of 10 days. Smears of the heated bacteria were made on cover glasses and stained with safranin in order to determine their staining qualities. These appeared to be similar to those of the nonheated crown-gall bacteria. Two series of inoculations were made with these materials: One with India ink, and another with a mixture of the ink and the heated organisms.

All of the substances mentioned in this and the previous paragraphs were introduced by mean of needle punctures into the upper parts of young tomato plants which were four to five inches high. A loopful of the material was placed on the surface of the stem and a needle thrust made through the center of the drop and then into the stem.

Material for study was collected at the following intervals of time after the treatment of the stems: 2, 15, 30, 60, 180, and 240 minutes. The fixative most commonly employed was formal-acetic-alcohol, but Flemming's solutions were also tried. The use of these fixatives destroyed the mitochondria present and avoided the possible confusion of these bodies with the bacteria. The fixed pieces of stems, which were 1 to 1.5 cm. long, were dehydrated in alcohol, treated with chloroform, and infiltrated and embedded in paraffin in the usual manner. Sections were made at varying thicknesses between eight and twenty microns; Flemming's triple stain was ordinarily employed. This stain was varied when it was desired to demon-

strate Gram-positive organisms by introducing Lugol's iodine solution following the treatment with gentian violet.

EXPERIMENTAL RESULTS

The efficiency of the various substances which were mixed with the crown-gall organism with the hope that they would indicate its location considerably exceeded the expectation. The material treated with mixtures of the crown-gall organism and Gram-positive organisms showed the location of the different kinds of bacteria very conspicuously. The dark purple of the masses of Gram-positive bacteria was frequently located by a hasty survey of the section under low powers of the microscope. Under high magnification the Gram-positive rods were easily observed. About these dark purple rods or intermingled with them the red rods of the Gram-negative crown-gall bacteria were discerned. The India ink also served admirably to facilitate the location of the organisms introduced with it. The black particles of the ink appeared in sharp contrast against all the rest of the slide. The comparatively hyaline or red rods of the crown-gall organism could be observed among the carbon particles. When used alone the ink showed the probable path the bacteria would likely follow. It was found that the Gram-negative crown-gall organisms could be obscured by the Gram-positive organisms or the ink particles of the concentration of these substances was proportionately too great. A preponderance of the crown-gall organism over the other substances employed was consequently shown to be desirable. Within the length of time employed none of the ingredients of a mixed suspension was found to separate one from another. This technique served not only to facilitate the location of the "zoogloeal masses" of the bacteria, and to follow their movement, but also to find small groups of bacteria or even individual organisms. Without the aid of such easily discernible bodies many of the smaller groups of the crown-gall organisms would certainly have been overlooked.

The substances used, whether employed alone or in combinations, were found to be located in the cavity made by the needle, in the ruptured cells about the cavity, in the intercellular spaces of the different tissues, and occasionally in the ruptured vascular elements. Occasionally, even in material fixed only two minutes after puncturing, the entire cavity made by the needle was occupied by the bacterial mass. So far, no bacteria or carbon particles have been observed inside uninjured cells, although ruptured cells were often completely filled. The chief avenue of progress from the puncture into the surrounding tissue was observed to be the intercellular spaces. Although intercellular spaces, wherever they occurred, appeared to be channels for the movement of the various substances, those of the pith and subepidermal region were the ones commonly followed.

The movement in the intercellular spaces was found to be chiefly up and down from the puncture with comparatively little lateral movement. Occasionally the organisms or carbon particles were found to have penetrated into the vessels of the xylem for considerable distance. However, this took place only following ruptures of the vessels by the needle.

Within the intercellular spaces of sections cut longitudinally the various bacteria and India-ink particles were found in three different types of distribution: viz, (1) as long continuous or irregularly broken masses resembling "zoogloeal strands," which sometimes filled the entire intercellular space and sometimes occupied only one portion of it; (2) as clumps of various sizes usually in contact with the cell walls but occasionally appearing to be free inside the intercellular space; and (3) as individual particles distributed within the liquid which flooded the intercellular spaces at the time of wounding. No one of these three types of distribution has been found to occur over the range of material studied in much greater percentage than the others. The types of distribution in small clumps or as individual organisms were found to be much less conspicuous than that in "zoogloeal strands." It is likely that, without the aid of the agents used to facilitate their location, many of the less conspicuous groups and the individual bacteria would have been overlooked entirely.

The appearance of the "zoolgoeal strands" was very conspicuous particularly in cases which involved the mixture of the crown-gall organisms with Gram-positive bacteria or with India ink. More or less densely packed masses of bacteria were readily observed inside the puncture and "intruding" along the intercellular spaces from the puncture. Within the intercellular spaces the "zoogloeal strands" were found in several conditions. In some cases the entire space was occupied. However, the strands sometimes appeared only on one side as if they had progressed only in one angle of the intercellular space. Cross sections have repeatedly shown that this does occur. Also in large spaces the strands sometimes appeared as though they had occupied the entire space but had been shrunk, as if by plasmolysis. They occupied either a lateral or a central position and left a considerable part of the space unoccupied. In such cases, particularly when ink was employed, traces of the strand were found on all the walls. When the advancing tip of the strand was found it often was characteristically convex. This rounded tip was a very conspicuous feature of these strands. It appeared similar to those described by Nixon (4) and Hill (3). It occurred in the cases of dead bacteria and India ink just as it appeared with living organisms.

Clumps of bacteria were observed which were of different sizes, irregular in shape, and commonly appeared to be attached to the walls about the intercellular spaces. In such cases the middle portion of the inter-

cellular spaces usually seemed to be clear of any visible material. These phenomena were observed also when India ink and a mixture of India ink and crown-gall bacteria were employed.

Individual bacteria were found widely distributed in the intercellular spaces and gave an appearance very unlike that of "zoogloeal strands." Their distribution was followed from the puncture out towards the end of the section for several millimeters but no distinct slime, membrane, or a definite tip was seen. As a rule, they appeared widely scattered in the liquid which flooded the intercellular spaces. The denseness of their distribution diminished gradually with the distance from the puncture until finally none could be located.

Cross sections of the material for study showed the bacteria and the ink particles to be situated in three typical positions. (1) As individual particles or small clumps adhering on all sides of the intercellular space without any visible membrane enveloping them, (2) as small wedge-shaped masses occupying one or more corners of an intercellular space, and (3) as large masses occupying the entire intercellular space. In the last two cases the bacteria were seen as small dots embedded in slime. The blunt ends of the wedged-shaped masses, in some cases, appeared concave but in others they were convex.

A slimy matrix appeared to be associated with considerable concentrations of bacteria. This slime was frequently found in the needle cavity which was sometimes occupied completely. Commonly it appears to have shrunk considerably and to have assumed a reticulate character following fixation and staining. It has also been observed often in the intercellular spaces where it appeared to enclose the bacteria. The composition of this slime is uncertain. However, it appears likely that the slime produced by the crown-gall organisms in culture and the cell contents released from the injured cells at the time of puncturing might provide the necessary materials. The consistency seems such that it could flow readily through the intercellular spaces under the influence of the forces existing there. Not infrequently hyaline areas similar to those described by Nixon (4) were found around the bacteria in this matrix. These areas were interpreted to be vacant spaces caused by shrinking during fixation similar to those found about bacteria stained in the background of milk or broth.

The rate of migration of the bacteria in the tissues of the plant was considered. For this purpose material for microscopic study was taken at different intervals, as has been stated earlier, beginning with material fixed immediately after inoculation and extending through four hours. The distances traveled by the bacteria were measured from the margin of the puncture to the most remote bacteria observed in the intercellular spaces. In figure 1, where the results of these measurements are charted,

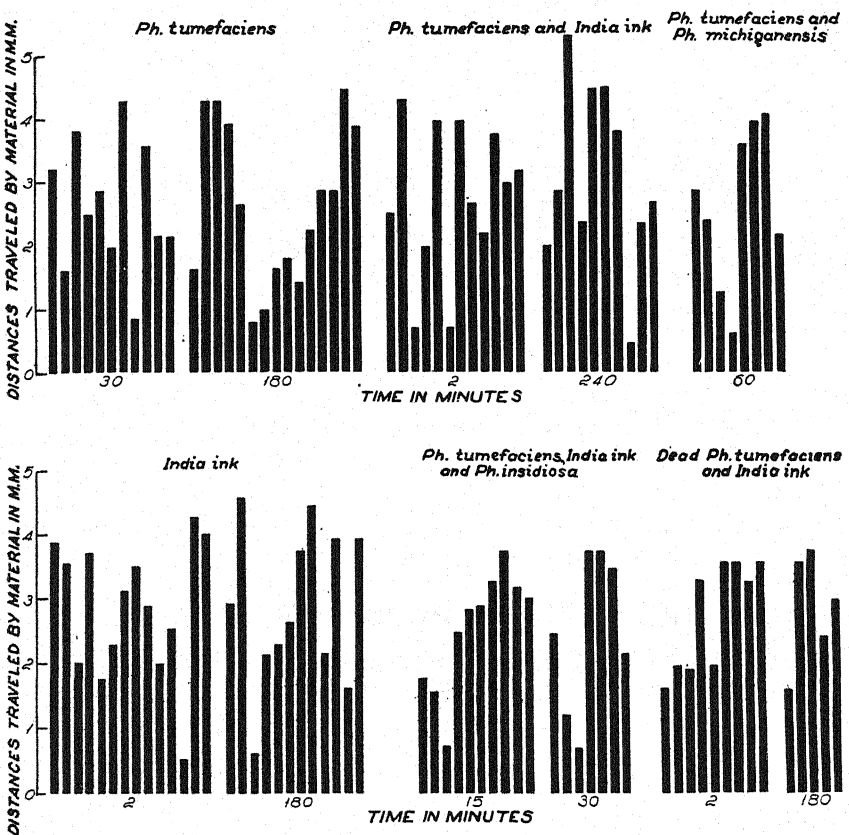


FIG. 1. Graphic presentation of measurements showing the distances traveled by various bacteria and inert materials, alone or in combination, in the intercellular spaces of tomato stems after stated intervals of time following their introduction through needle punctures.

it may be seen that there are no significant differences in the average distances traversed by the bacteria in the various time intervals. A summary of these measurements, showing the distances traveled by various bacteria and inert substances in the intercellular spaces after stated intervals of time, is given in table 1.

The trials performed with dead bacteria, India ink, or a mixture of both offer important evidence on the movement of the various substances in the intercellular spaces. These nonliving particles appeared in essentially the same distributions as the living bacteria. They formed similar strands and masses (Fig. 2, A–E) and were found also in small groups or as isolated particles. As in the cases with the living bacteria, there were no great differences in the average distances traversed during the various time intervals.

TABLE 1.—*Summary of measurements showing the distances traveled by bacteria and inert materials in the intercellular spaces of tomato stems after stated intervals of time following their introduction through needle punctures*

Materials introduced	Time between inoculation and fixation	Measurements	Range of distance moved	Average distance moved
	Minutes	No.	Mm.	Mm.
	30	11	0.83 to 2.24	2.61
Ph. tumefaciens	180	15	0.80 to 4.42	2.68
Ph. tumefaciens and India ink	2	12	0.70 to 3.90	2.76
	240	10	0.44 to 5.31	3.04
Ph. tumefaciens and Ph. michiganensis	60	8	0.61 to 4.07	2.59
Ph. tumefaciens, Ph. insidiosa, and India ink	15	10	1.72 to 3.71	2.54
	30	7	0.71 to 3.70	2.48
Dead Ph. tumefaciens and India ink	2	9	1.59 to 3.54	2.72
	180	5	1.59 to 3.54	2.86
India ink	2	14	0.53 to 4.24	2.92
	180	12	0.60 to 4.42	2.90

Experiments on the possible importance of capillarity were made in relation to the intercellular spaces. Small capillary tubes of glass were drawn to a size corresponding to intercellular spaces. The suspensions of bacteria and inert substances rose in them quite rapidly. The top of the column was always concave when the liquid rose under the influence of capillarity. However, when sufficient negative pressure was supplied, the top of the liquid was convex. The use of these tubes was open to objection because they provided a cylindrical column, while the intercellular air spaces usually possess several hollow wedges. Consequently, glass rods of a suitable size were drawn out and arranged in small bundles. The rise of liquid between them was studied. The meniscus was concave or convex, respectively, depending on conditions, just as it had been with the capillary tubes. However, because the rise was difficult to follow clearly when the rods were employed in bundles, they were laid as individuals upon long cover glasses so that they were in contact with the cover glasses for some distance. The cover glasses were stood on end, and a small drop of the liquid being studied was placed at the lower end. Its rise in the hollow wedges between the round rods and flat glass was followed with a low-power microscope by looking through the cover glass. As was to be ex-

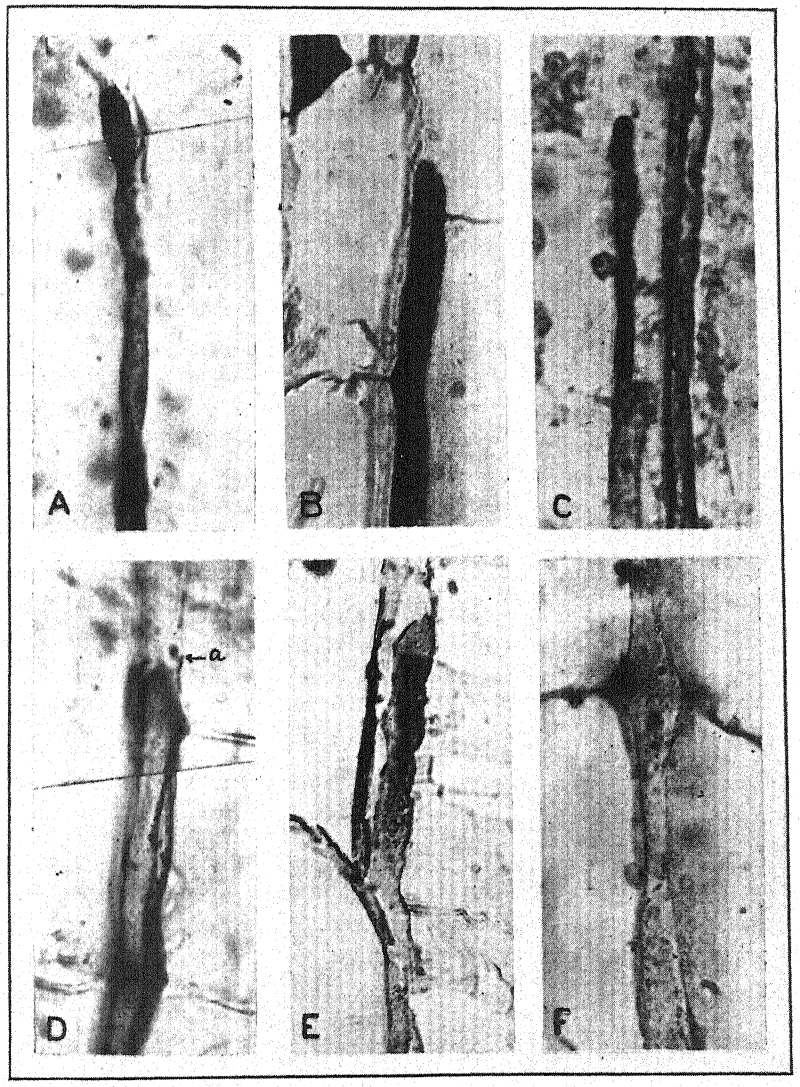


FIG. 2. Photomicrographs of Burri's India ink, crown-gall bacteria, and a mixture of both in the intercellular spaces of tomato stems. Magnification approximately $\times 1000$.

A and B, Strands with convex ends of India ink.

C, D, and E, Strands with convex ends of a mixture of India ink and killed crown-gall bacteria. The bacteria and ink show more or less irregular distribution, sometimes appearing as clumps on the walls. In D particles of ink appear at *a* on the walls a short distance above the convex end of the strand. This suggests that the strand once stood at a higher position. Perhaps plasmolysis accounts for the withdrawal downward of this strand and the change from a concave to a convex shape at the tip. It probably accounts also for the clear spaces at the sides of the strand in both D and E.

F, Free crown-gall bacteria in the liquid released into the intercellular spaces by the puncture.

pected, the liquid rose first in the apical angle of the wedge between the glass rod and cover glass. The advancing tip representing the raised edge of the meniscus in the narowest part of the angle was ordinarily pointed, but sometimes when it stopped the liquid crept along the cover glass and appeared to form a more or less rounded tip. An attempt to provide small irregular spaces more like those in the plant was made by fusing grains of white sand, sifted to secure the desired size, to a cover glass. This was mounted under a microscope, cover glass up, and a small drop of the liquid placed at the edge. The movement was similar in the irregular space to that between the glass rod and cover glass. The rapidity of the movement in all these cases was influenced by the density of the bacterial suspension. When the bacterial slime was transferred without dilution directly from an agar slant the movement was much slower than in the case of a water suspension of the bacteria. This difference in speed appears to have been due to a difference in viscosity.

Although the subject of this study has been primarily the movement of the crown-gall bacteria during the early hours after their introduction into the stem of the tomato plant, a few sections were made through galls two to three weeks old. The technique employed was the same as in the case with the work on the early migration of the bacteria. The results secured have confirmed those reported earlier by Riker (5) and by Robinson and Walkden (7) that the organisms are located within the intercellular spaces and that they exert a cell-stimulating influence from that position.

DISCUSSION

The Gram-positive bacteria and Burri's India ink have been valuable aids, when mixed with cultures of the crown-gall organism, in locating this organism within the host tissue. In actual practice such mixtures were of much greater assistance than was expected. However, this technique is open to the objection that these substances, when mixed with the crown-gall organism, may provide a condition different from that in which the crown-gall organism is used alone. In both these cases the condition may also be different from that found during natural inoculation in the field. However, it appears unlikely that during the short time involved in these experiments, the Gram-positive bacteria and India ink have introduced any important error. On the other hand, they have increased so greatly the possibility of locating and observing the crown-gall bacteria that their use appears very advantageous.

The observations herein reported confirm those of Robinson and Walkden (7) and Hill (3) that the bacteria occur in masses embedded in slime which they call "zoogloeae" and "zoogloeal strands." However, this is not the only form in which the bacteria appear in the early stages after in-

oculation. Small clumps, as well as individual bacteria, have been found commonly to occupy intercellular spaces several millimeters long. These findings emphasize Riker's (5) earlier report of the importance of the liquid which flooded the intercellular spaces following the rupture of the cells in the path of the inoculating needle. In this connection it is noteworthy that this liquid contains the entire cell contents and not merely the cell sap. Consequently, a considerable part of it is not subject to reabsorption into the surrounding tissue as suggested by Hill (3).

The conception that the movement of the organisms through the host tissue is due to growth has not found support in these studies. Four lines of evidence oppose this idea. (a) No significant progressive movement has been found for the different time intervals. (b) Such inert substances as dead bacteria and India-ink particles have shown a similar movement to corresponding distances. (c) The lag period, found by Wright, Hendrickson, and Riker (8) following transfers of the crown-gall organism, indicates that vigorous growth does not occur until considerably later than the times considered in this work. (d) The earlier report by Riker (5) that the galls developed within the general region involved by flooded intercellular spaces after puncturing has been repeated and confirmed many times. If the crown-gall organism were capable of active migration by growth, the crown galls which follow puncture inoculations would probably not be so definitely limited.

The mechanism of the movement of the bacteria is not definitely understood. Since it appears that growth has little or nothing to do with this movement within a few hours after treatment, it seems that various physical forces, including capillarity, convection currents, and negative pressure, may be responsible. Under the conditions of these experiments, motility by its flagella of the crown-gall organism seems to have little to do with the progress of the organism in the tissue except, perhaps, in the cases of isolated bacteria. The rapidity of the movement first suggests that either negative pressure or capillarity is primarily responsible for this movement. Under the influence of negative pressure a convex tip would be expected on "zoogloeal strands." It is possible that, after rising by capillarity, the concave tip of a "zoogloeal strand" may become somewhat "plasmolized" during the process of preparing the material for examination and change into a convex form. It is well known that when cell contents become plasmolized they withdraw from the corners of the cell walls and commonly appear comparatively convex, rather than angular according to the form of the wall. That such a thing does at times take place in the case of bacterial masses in the intercellular spaces is suggested by a number of tips, one of which is shown in figure 2, D. Particles of ink may be seen adher-

ing to the wall beyond the tip of the strand. In this case it is probable that plasmolysis has turned a concave tip into one that is convex. It seems unlikely that such details could be observed when the crown-gall bacteria are employed without the addition of the ink. In this same figure there also is evidence at the side of the strands that shrinkage has occurred.

The collection on the intercellular walls of thin layers of bacteria and clumps of various sizes, leaving the middle portion of the intercellular space free, may also be influenced by physical forces, including shrinkage. It is considered that originally these bacteria were more or less evenly distributed in the liquid but under the influence of different reagents it shrunk, accumulated upon the cell walls, and left the middle of the intercellular space apparently free.

In consideration of the available evidence it appears that, instead of growth, various physical factors, including perhaps water content of the host plant, release of cell contents by the needle puncture, bleeding of the host plant, capillarity, diffusion and negative pressure, may influence the movement of the crown-gall bacteria and the manner of their distribution within a few hours after inoculation under the conditions studied. The form of these strands as they appear in histological preparations, however, may depend upon the shape of the intercellular space and the degree of shrinkage. Later on, growth and metabolism of the organism and the various physiological responses of the host plant, together with these physical factors, may be influential in the progress of the bacteria through the tissue. In this connection osmotic pressure must certainly be taken into account. A consideration of this phase of the subject is beyond the scope of this paper.

SUMMARY

1. Finding the crown-gall bacteria in the stems of tomato plants a few hours after their introduction through needle punctures was facilitated by mixing Gram-positive bacteria or Burri's India ink with the inoculum.

2. The bacteria were found in the cavity made by the needle, in ruptured cells, and in the intercellular spaces. They occurred in the stained preparations as strands which were sometimes continuous and which had convex tips, as clumps of a number of individuals, and as individual cells. All three types of distribution were observed in the same slide.

3. The distances traveled by the bacteria within the first four hours after introduction were quite variable. No significant progressive movement attributable to growth was observed.

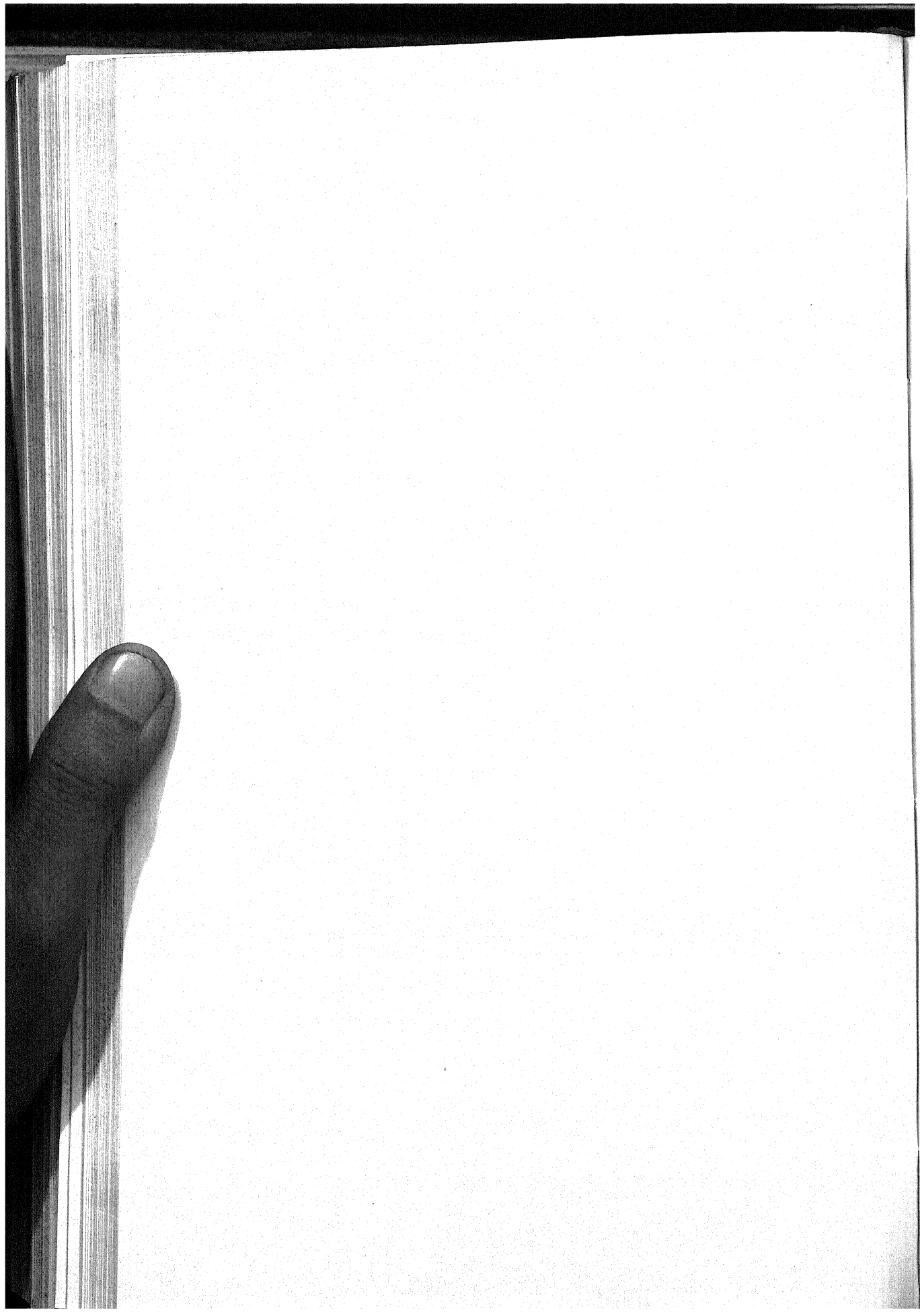
4. Dead crown-gall bacteria and particles of India ink showed the same types of distribution and moved approximately the same distances as the living crown-gall bacteria.

5. The mechanism by which the bacteria and inert substances employed in these experiments are moved through the intercellular spaces is not definitely understood. Several lines of evidence oppose the concept that the movement studied was the result of growth. Various physical forces, particularly capillarity and negative pressure, seem important. The physiological disturbances incident to the inoculations also deserve consideration.

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SOME POSSIBLE CAUSES OF STREAK IN TOMATOES[1]

W. D. Valleau and E. M. Johnson

In studying the problem of the virus diseases of tobacco, as they occur in Kentucky, one is impressed by the increasing number of what appear to be distinct strains of several groups of viruses. The diseases produced by the different viruses often can be distinguished only by a comparative study under controlled conditions. Several of the tobacco viruses under study are likewise found in tomatoes, causing a disease which might logically be called tomato mosaic. Therefore the terms tobacco mosaic and tomato mosaic have but little meaning to a student of the virus diseases of these crops and merely lead to confusion when used in this way in the literature. This statement may need some qualification with respect to true tobacco mosaic, typical cases of which are recognized by most pathologists, but even true tobacco mosaic may be caused by several distinct virus strains. It now appears to be agreed that tomato streak is caused by a virus or a mixture of viruses but there seems to be doubt as to the exact identity of the viruses concerned. One of them is the virus present in the so-called healthy potatoes. The identity of the others, if more than one is concerned, is certainly a matter of conjecture. As the writers have a considerable number of distinct virus diseases under study, practically all of which were obtained originally from naturally infected tobacco, an attempt was made to clarify some of the confusion which appears to exist as to the cause of streak in tomatoes.

LITERATURE REVIEW

As the literature on streak has been reviewed recently (1, 9) it is hardly necessary to mention more than a few recent papers. Gardner and Kendrick (2), in discussing mosaic of tomatoes, pictured streak as one of its symptoms, thus recognizing the virus nature of the disease. Vanterpool (9) observed the significant fact that if streak material is dried from 3 to 9 months and then inoculated into tomato plants, it no longer produces streak but only a severe case of mosaic. He thus demonstrated that a strain of true tobacco mosaic was present. Acting on the suggestion obtained from a report by Johnson (3), he inoculated tomato plants with tomato mosaic and an extract from mosaic potatoes and obtained what he considered to be typical streak.

Berkeley (1), while admitting the virus nature of streak, states that "the experimental evidence reported here does not agree with the theory

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

of a combination of tomato and potato mosaic viruses as the true cause of streak." He claimed that juice from healthy potato plants produced definite streak in tomatoes, as well as that from mosaic potato plants, and from potatoes with streak. Vanterpool had been unable to produce streak in tomatoes by inoculating with potato streak material. Berkeley also noticed apparent differences in rate and ease of spread of streak in various places and likewise differences in symptoms, which appeared to be of significance. Smith (5), in studying the transmission of a potato-mosaic virus to tomatoes, found that a mixture of the potato virus with a yellow mosaic will cause a severe disease which has all of the characteristics of streak. The mixed-virus theory of the disease is also confirmed by Stover (6), who showed that mosaic tomato plants developed streak when inoculated with the juice of potatoes affected with mild mosaic, rugose mosaic, leaf roll, spindle tuber, or apparently healthy potato. While there appears to be a multiple cause of streak in these experiments, all may be interpreted as confirming the tomato mosaic plus healthy-potato virus theory of the disease, as it is entirely probable that the various potato plants used all carried the healthy-potato virus in addition to some other virus, the latter either alone or in conjunction with the former resulting in the symptoms described as rugose mosaic, leaf roll, etc.

FIVE GROUPS OF VIRUSES AFFECTING BOTH TOBACCO AND TOMATO

For the past several years the writers have been studying the virus diseases of tobacco as they occur on the Experiment Station Farm at Lexington and vicinity and have obtained a few tobacco viruses from other States (7). The Experiment Station Farm is particularly well supplied with virus diseases, probably because potatoes, tobacco, and tomatoes have been grown more or less regularly for many years and because perennial weed hosts are abundant. Cultures of all the virus diseases which in any way appear to differ have been collected and then passed through a succession of Turkish tobacco plants in the greenhouse and the symptoms compared. In this way it has been possible to separate virus diseases which superficially appear alike and to group them on the basis of similarities of symptoms. At least five groups of viruses that affect both tobacco and tomatoes have been recognized. These are the true tobacco-mosaic group, characterized by the well-known symptoms of tobacco mosaic, having the unique ability of living for years in dried tobacco material, and apparently affecting only members of the Solanaceae; the etch group, characterized by a fine necrotic etching on the rubbed leaves and on one or more leaves which first develop after inoculating Turkish tobacco plants; the viruses of the cucumber-mosaic group, best characterized by their ability to cause mosaic in

cucumbers (tobacco ring spot will also do this but should not be confused with the cucumber mosaics); the veinbanding virus, causing a disease in tobacco fields where potatoes have formerly been grown but causing very faint symptoms in tomato; and the virus obtained from so-called healthy potato, which will be designated as healthy-potato or the potato virus in this paper. The disease caused by the healthy-potato virus used in these experiments is of the type designated by James Johnson (4) as "ringspot."

The studies to be reported have been conducted with certain of the above-mentioned virus strains which were thought to be pure or to contain but a single virus strain. It must be stated that there is no absolute certainty on this point, but it is probable that if mixtures occurred they were between strains of the same type of virus so much alike as not to affect the results. Small numbers of plants have been used throughout the tests, a fact which might bring their accuracy into question, but, as the methods of handling the virus diseases have been refined over a period of years to a point where accidental infections rarely occur and as transfers have been made back to tobacco plants in all cases, we have confidence in the results. The method of inoculation used is that of crushing the viruliferous material in a sterile culture dish with a stick bound on the end with a piece of cheesecloth and then sterilized. A piece of waxed paper is laid on the left hand, the leaf to be inoculated is supported on this paper, and the padded stick used to rub the inoculum on the leaf. Although many virus strains are kept in one house, accidental infections are so rare as to be nearly negligible.

The tomato plants were grown in half gallon or two gallon jars of forest soil and were kept in a vigorous growing condition by frequent applications of nitrogen salts.

The healthy-potato virus used in two experiments was obtained from a very mildly mottled tomato plant growing in the Horticultural Greenhouse not far from Irish Cobbler potatoes. This virus strain had been studied for over a year in comparison with the virus obtained directly from these Cobblers, and no differences could be noted in Turkish tobacco plants infected with them. In a third experiment the virus used was obtained originally from an apparently healthy Irish Cobbler plant.

TOBACCO MOSAIC VIRUSES PLUS HEALTHY-POTATO VIRUS CAUSE STREAK

Tomato plants were inoculated with the potato virus, with three distinct strains of true tobacco mosaic, and with these in combination with the potato virus. Mild mosaic alone caused very mild symptoms, the plants appearing nearly healthy. Severe mosaic caused leaf distortion, blistering, mottling and no necrosis. The healthy potato virus alone caused faint mot-

tling but no distortion or necrosis. In combination with healthy-potato virus mild mosaic resulted in a mild form of stem streak with leaf necrosis, faint mottling, and slight leaf distortion. The potato virus in combination with severe mosaic resulted in streak of a more severe form. Stem and petiole streaks were numerous and prominent, leaf necrosis was severe and the leaves were distinctly mottled and distorted. Yellow tobacco mosaic, a disease very similar to severe tobacco mosaic except that yellow blotches are produced in the leaves, in combination with the potato virus caused streak of about the same degree of severity as that resulting from severe tobacco mosaic in combination with the potato virus. In tomato the yellow blotches are produced as in tobacco.

Inoculations were made from the affected tomato plants to Turkish tobacco. The combination viruses in each case produced symptoms typical for the tobacco virus concerned except that small necrotic rings typical of the potato virus usually developed on rubbed leaves and small necrotic spots developed on the later mottled leaves. The single viruses in each case gave the expected results.

CUCUMBER MOSAIC VIRUSES PLUS HEALTHY-POTATO VIRUS CAUSE STREAK

Tomato plants were inoculated with three strains of cucumber-mosaic (Types 1, 2, and 3) which produced distinct symptoms on both tobacco and cucumbers, and with these in combination with the potato virus. Type 1, which represents the cucumber mosaic most commonly found in cucumbers and tobacco in Kentucky, caused narrowing of tomato leaves but no necrosis except on rubbed leaves. Type 2 caused no leaf distortion or necrosis but the leaves produced subsequent to inoculation were mottled with ring-like patterns. Cucumber mosaic type 3 causes severe distortion in tobacco and results in fern-leaf and slight leaf necrosis of unrubbed leaves in tomato. While the cucumber mosaics inoculated in combination with the potato virus did not produce streak so consistently as the true tobacco mosaics yet streak was produced with each of the three types. Failure of the cucumber mosaics to produce streak in certain instances appeared to be due to difficulty in transferring these viruses to tomato. Cucumber mosaic type 3 plus the potato virus produced more severe streak symptoms than either of the other two types and in one experiment this virus combination produced more severe symptoms than any other combination tested. In one instance the fruit on a streak plant was spotted with small brown raised lesions on the surface of the fruit. This was the only case observed where the fruits showed symptoms except those on plants affected with ring mosaic. The absence of fruit symptoms is probably of little significance as the plants could not be kept in as vigorous a state of growth in jars as in

benches of soil. Inoculations from the affected tomato plants to tobacco indicated that accidental infections with the true tobacco mosaic viruses had not occurred in the case of streaked tomato plants, although one tomato plant inoculated with cucumber mosaic type 3 alone was found to have contracted tobacco mosaic toward the end of the experiment. Tobacco plants affected with the mixed viruses, cucumber mosaic and healthy potato, developed symptoms typical of the cucumber mosaic concerned but with some leaf necrosis.

ETCH VIRUSES PLUS HEALTHY-POTATO VIRUS CAUSE STREAK

Tomato plants were inoculated with three strains of the etch virus, etch, etch +, and severe etch, each capable of causing a disease of a different degree of severity in tobacco. These were also used in combination with the healthy-potato virus. The etch viruses alone cause narrowing, marked distortion and mottling of tomato leaves. The diseases produced could readily be mistaken for those produced by the more severe strains of tobacco mosaic. Plants affected with severe etch were always more stunted than other plants in the experiments. Each of the etch viruses in combination with the healthy-potato virus caused stem and petiole streak and extensive leaf necrosis. Etch +, which produces more marked symptoms in tobacco than etch, caused more severe streak on tomato when combined with the potato virus than etch. Transfers from the affected tomato plants to tobacco gave the expected results indicating that accidental infection with tobacco mosaic had not occurred. The potato virus caused slightly more pronounced necrosis in tobacco when combined with an etch virus than the etch virus alone.

RING MOSAIC VIRUS USED ALONE PRODUCES STREAK[2]

The ring mosaic virus, so named because it produces symptoms somewhat similar to tobacco mosaics but with a distinct tendency for the mottling to be in the form of rings, appears to be of the same nature as the true tobacco mosaics in that it withstands drying. This virus in combination with the healthy-potato virus causes a severe stem and petiole streak and leaf necrosis. When used alone it also causes stem and petiole streak and leaf necrosis, but perhaps slightly less severe than is caused by the mixture. The virus has produced streak in tomatoes when transferred either from green tobacco plants or from dried tobacco. As the healthy potato virus does not appear to be able to withstand drying the possibility of a mixture with it seems to be eliminated. Fruits produced on tomato plants affected with ring mosaic often develop depressed chlorotic rings of varying size. The rings sometimes become necrotic soon after they develop resulting in

[2] This virus disease of tobacco was earlier designated C.T. (6).

misshapen distorted fruits. Transfers from the affected tomato plants to tobacco gave the expected results, with slightly increased leaf necrosis in the case of the mixture.

VIRUS COMBINATIONS WHICH DID NOT PRODUCE STREAK

All of the possible combinations of the different strains of viruses under study were not tested for their effect on tomatoes. The possible combinations of virus groups, except vein banding, were made but none in which the healthy-potato virus was not present produced streak or leaf necrosis. The combinations tested were: Cucumber mosaic type 3 plus severe tobacco mosaic, cucumber mosaic type 2 plus etch +, and etch + plus mild tobacco mosaic. A combination of veinbanding with the healthy-potato virus failed to produce streak in tomatoes although it sometimes results in slight-leaf necrosis. This combination frequently causes streak in seedling potatoes and the veinbanding virus alone when passed to Cobbler potatoes frequently causes leaf necrosis and streak, although the latter symptoms usually disappear if the plants make further growth.

DISCUSSION

The results have shown clearly that streak of tomatoes may be caused by each of the virus strains tested except veinbanding, when used in combination with the virus from apparently healthy potatoes. One virus of the true tobacco-mosaic group, designated ring mosaic, was found to produce streak when used alone. It produces depressed, somewhat chlorotic rings sometimes half an inch in diameter on the surface of the tomato fruits, and can thus be easily identified.[3] Ten cases of tomato streak were produced by inoculating 10 tomato plants with dried tissue of plants infected with this virus, thus tending to eliminate the possibility of its being a mixture with other than true tobacco mosaics.

While all possible combinations of the virus strains were not tested, the possible group combinations, except veinbanding, were tried with the result that no combination in which the potato virus was not present produced streak.

It is thus seen that streak is a symptom with little or no etiologic significance except that it indicates a virus disease of tomatoes due, in certain instances, to a mixture of viruses, one of which is likely to be the virus com-

[3] This disease of tomatoes is apparently identical with one of the streak diseases reported at the Des Moines meeting by S. P. Doolittle and H. L. Blood (Abstract in Phytopath. 20, 1930) in which similar rings on the fruit were illustrated. Doctors Max W. Gardner and J. B. Kendrick informed the writers that similarly marked fruit is often seen in shipments of tomatoes from California. Inoculations from what may have been one of these fruits was reported on in Brewer, Kendrick and Gardner. Effect of mosaic on carbohydrate content of the tomato plant. Phytopathology 16: 843–851. 1926.

monly found in apparently healthy potatoes. This raises the question as to the cause of streak in epiphytotics. That it will be found to be caused by different viruses or mixtures of viruses is to be expected from the published reports of other investigators. Berkeley claimed to be able to produce streak with the juice of healthy, mosaic, and streak potato plants. The cucumber-mosaic viruses and the etch viruses are each capable of infecting potatoes and may have been concerned in Berkeley's experiments, or it is not impossible that viruses other than those so far recognized may have been present in his potatoes and, together with the healthy-potato virus, may have caused streak when transferred to tomatoes. Considering the large number of virus diseases of potatoes and the almost universal presence of at least one virus in all apparently healthy potatoes, it would not be surprising if, even in seemingly healthy potatoes of certain varieties, a virus combination were found, capable of causing streak in tomatoes. In the present studies we have seen no indication that the virus commonly present in healthy potatoes, when used alone, can cause other than a mild mottling in tomatoes.

In epiphytotics of streak in tomatoes, it is desirable that the particular viruses concerned be determined, as this knowledge may assist in determining the source of the trouble. If only tomatoes are used as a host for a study of the virus symptoms, determinations are nearly impossible. Turkish tobacco has proved valuable for this purpose because the plants are relatively small and occupy but little bench space and because the symptoms of the various virus groups are distinct enough to make determinations comparatively easy. If transfers are made from streaked tomato plants to Turkish tobacco by the rubbing method, both viruses concerned are likely to be transmitted to the tobacco. Usually the healthy-potato virus will manifest itself by the presence of small necrotic rings on the rubbed leaves and by a slightly increased amount of necrosis in later leaves, if it is in combination with another virus. The other virus will usually produce nearly its typical symptoms, which may be recognized. For those not familiar with the symptoms of the virus groups here discussed, further tests may be necessary.[4] If the streak material is dried and inoculations of tomato or tobacco plants produce a mosaic, it is almost certain to be one of the strains of true tobacco mosaic. For comparison, true tobacco mosaic may be readily obtained by moistening tobacco of nearly any of the common brands of cigarettes or smoking tobaccos and rubbing this decoction on healthy tobacco leaves. If the virus from streak plants does not withstand drying, it may be the virus of one of the cucumber mosaics. This may be deter-

[4] A more extensive description of the virus diseases concerned in the present studies will be found in Kentucky Exp. Sta. Bul. 306.

mined by inoculating cucumbers. If mosaic develops, it may be considered proof that one of the strains of cucumber-mosaic virus is involved, as at present there is only one other known cause of mosaic in cucumbers, namely, tobacco ringspot, and this will not transfer readily to tomatoes. If one of the etch group of viruses is concerned, the fact will readily be recognized if young, vigorously growing Turkish tobacco plants are inoculated with the streak tomato juice. The fine necrotic etchings which appear on the upper surface of one or more of the new leaves of Turkish tobacco following inoculation are very characteristic. In view of the fact that members of these virus groups are capable of producing streak on tomatoes when inoculated together with the potato virus, it would not be surprising if other viruses also may be found to cause streak of tomatoes when mixed with the healthy-potato virus.

A determination of the viruses concerned in streak may suggest its origin and consequently may assist in control. If the healthy-potato virus is one of the complex, it immediately suggests potatoes as at least one source. If the true tobacco mosaic is one of the complex, in a region where tobacco is not commonly grown, personally used tobacco is suggested as a possible source of this virus, as it is readily transmitted to tomatoes if the hands become contaminated by handling viruliferous smoking or chewing tobacco; or they may become contaminated by handling unheated tobacco stems, commonly used for fumigating greenhouses.

The cucumber-mosaic viruses and the etch viruses may originally have come from potatoes, as both appear to be transmissible to and from potatoes by insects or they may come from weeds (8). It has been observed that one of the etch viruses will spread very rapidly through a tomato planting in the field if a source of inoculum is present.

The seeming failure of *Myzus persicae* to transmit the healthy-potato virus[5] suggests that its presence in tomatoes in the greenhouse may result from handling potatoes or potato plants carrying the virus and subsequently handling tomato plants. It thus appears that mechanical as well as insect transmission must be given consideration in attempting to explain original infections of tomatoes with streak and its subsequent spread.

CONCLUSIONS

1. A study has been made of the possibility of causing streak in tomatoes with various tobacco viruses, both in combination with the so-called

[5] This is based on the belief that the ringspot of tobacco, as reported by K. M. Smith (Ann. Appl. Biol. XVI, No. 3, 1929) is caused by a mixture of the healthy-potato virus and his insect-transmitted virus, which produces a disease in tobacco which we have previously named veinborder or veinbanding. Smith has assumed that apparently healthy potato plants are virus-free because of h's failure, following inoculation to tobacco, to obtain recognizable symptoms.

healthy-potato virus and in other combinations in which the healthy-potato virus was not included.

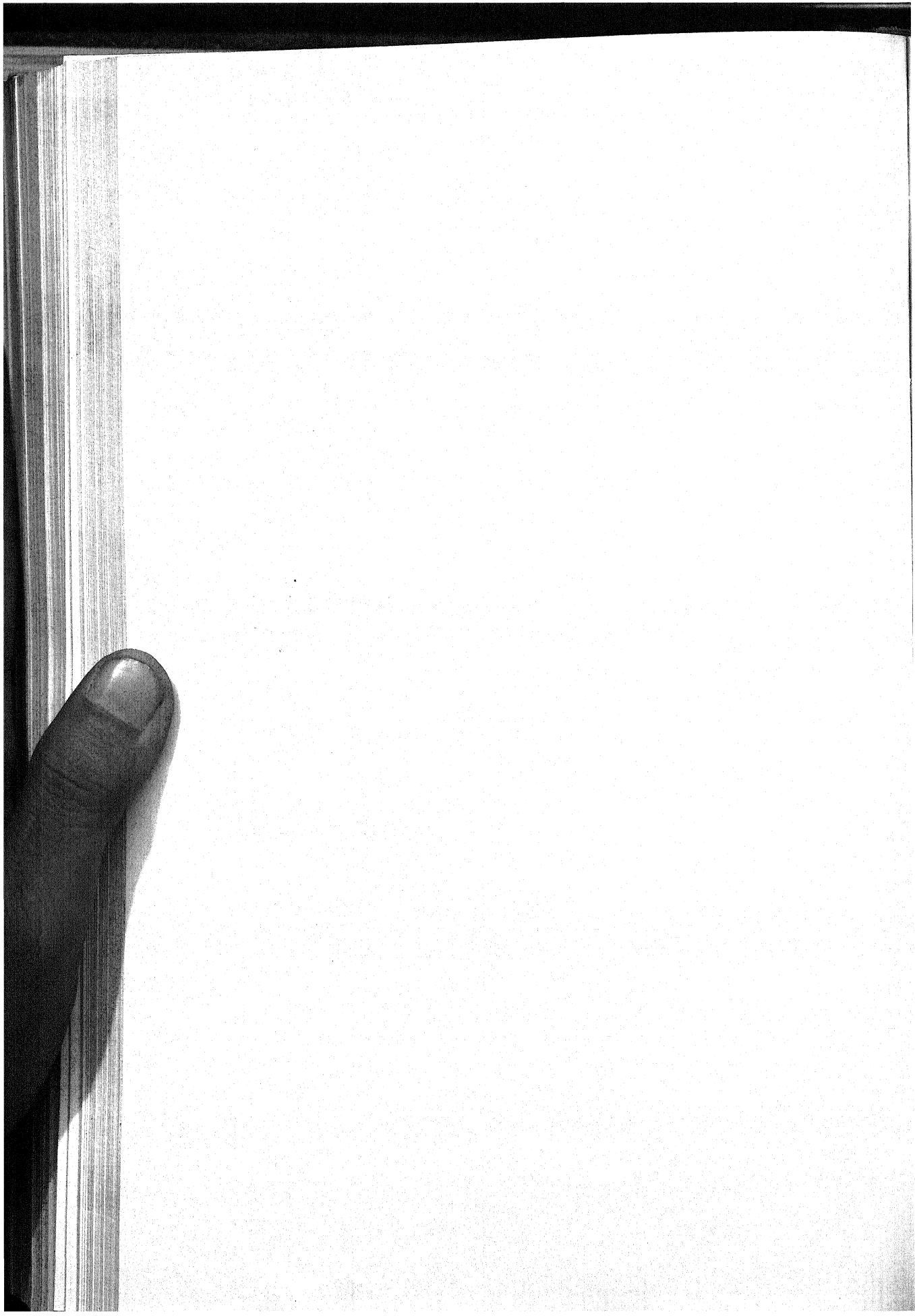
2. Streak has been produced by an unmixed virus apparently belonging to the true tobacco-mosaic-virus group; by three other strains of the true tobacco-mosaic viruses plus the healthy-potato virus; and by three strains of the cucumber-mosaic viruses plus the healthy-potato virus; and by three strains of the etch virus plus the healthy-potato virus.

3. Streak was not produced by a mixture of one of the etch viruses and a virus of true tobacco mosaic, by the cucumber-mosaic virus plus true tobacco-mosaic, by cucumber-mosaic virus plus the etch + virus, or by the healthy-potato virus plus veinbanding.

4. The virus strains within a given virus group differ primarily in severity of symptoms produced on a given host. In the three virus groups under study, the one in each group producing the most severe symptoms on tobacco resulted in the most severe streak of tomatoes when combined with the potato virus.

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THE LONGEVITY OF PHYLLOSTICTA SOLITARIA E. AND E. ON APPLE SEEDLINGS HELD IN COLD STORAGE

J. A. McCLINTOCK

Gardner and Jackson[1] have pointed out the relationship of blotched apple seedlings to the spread of *Phyllosticta solitaria* in Indiana, and observations in Tennessee since 1923 fully substantiate their findings.

Since most of the blotch cankers on apple seedlings are above the crown, it is to be expected that there will be less spread of the causal fungus from infected seedlings to scions in nurseries where most of the propagation is done by whip grafting. Nearly all of the seedling top is discarded in making piece-root grafts, yet the danger from blotch-fungus-infected seedlings is not entirely eliminated, as is shown by cases of well-developed blotch cankers on the trunks of grafted apple trees, both below and above the union.

Examination of apple seedlings shipped into Tennessee since 1923 has shown the presence of varying amounts of blotch-fungus infection from year to year. Since blotch-infected seedlings are found continually in shipments, nurserymen should cull, on arrival from seedling growers or dealers, all trees which show such cankers.

APPLE BLOTCH ON SEEDLINGS FROM COLD STORAGE

The extent to which fruit-tree seedlings are held in a dormant state, from one year to another, in cold storage is not definitely known. However, two cases are known where, in years of heavy seedling production, a Tennessee nursery held surplus lots of 50,000 and 60,000 apple seedlings in cold storage throughout an entire growing season and then used them the following year.

The apple seedlings from which the following data were obtained were grown in midwestern seedling nurseries in 1927 and shipped to the purchaser in the winter of 1927–28. They were held in paper-lined boxes in nonheated rooms until May 1, 1928. Then 60,000, in their original packing boxes, were placed in a commercial cold storage. They were held at temperatures ranging from 34° to 36° F. No moisture was added during the storage period. On February 8, 1929, the boxes of seedlings were removed after being in cold storage for nine months. They were taken to the nursery to be used for propagating purposes.

A few hundred of these stored seedlings were obtained for tests at the Tennessee Agricultural Experiment Station in comparison with seedlings

[1] Gardner, Max W., and H. S. Jackson. New aspects of apple blotch control. Phytopath. 13: 44. 1923.

grown in 1928. While examining these cold-storage seedlings, preparatory to lining out in test rows at the University farm, the writer observed typical blotch cankers. This test lot was then carefully sorted and the seedlings having the largest number of cankers per tree were selecteed. The cankers appeared similar to those found from year to year on seedlings raised in midwestern nurseries. Careful examination of each canker showed no evidence of enlargement of the original cankers during the cold-storage period of nine months.

THE DEVELOPMENT OF BLOTCH-FUNGUS CANKERS ON POTTED SEEDLINGS

According to Roberts,[2] the blotch-fungus in the older portion of a canker dies. As these stored seedlings showed no evidence of canker growth for more than a year, it seemed desirable to determine whether the cold-storage period to which the seedlings had been subjected had eliminated the danger of spreading *Phyllosticta solitaria* from the cankers formed during 1927. Seedlings from the most heavily infected lot were root pruned, and set in large pots of soil on March 8, 1929. Among these potted seedlings were planted apple seeds freshly removed from healthy Yates apple fruits. The pots were labeled and set on a greenhouse bench for observation. Buds on some of the stored seedlings began to show signs of growth within three days and, in most cases, the seedlings developed normally. Some seedlings in each pot failed to start and within a few weeks died. Somewhat later additional seedlings appeared in the pots from the Yates seed planted March 8, 1929.

As the season advanced, temperatures within the greenhouse became too high for the best growth of the seedlings, so the pots were moved to a plat outside the greenhouse but near enough to it to be watered. As seen in figure 1, these potted seedlings made fair growth, considering the restricted soil area to which they were confined.

As the growing reason advanced, it was obvious that the blotch fungus was spreading from the old cankers into the surrounding stem tissues of the potted seedlings. Eventually pycnidia appeared on the surface of the new canker tissues formed about the old cankers. This indicated that *Phyllosticta solitaria* in cankers formed on the seedlings in the midwestern nurseries in the summer of 1927 had remained alive but dormant until conditions became again favorable for its development in the spring of 1929. Following the development of new canker growth on the stored seedlings, as seen in figure 2, *1* typical blotch-fungus infections began to appear on the leaves, which were chiefly on the new growth above the cankers. In contrast the leaves on the small Yates seedlings were heavily

[2] Roberts, John W. Apple blotch and its control. U. S. Dept. Agr. Bul. 534. 1917.

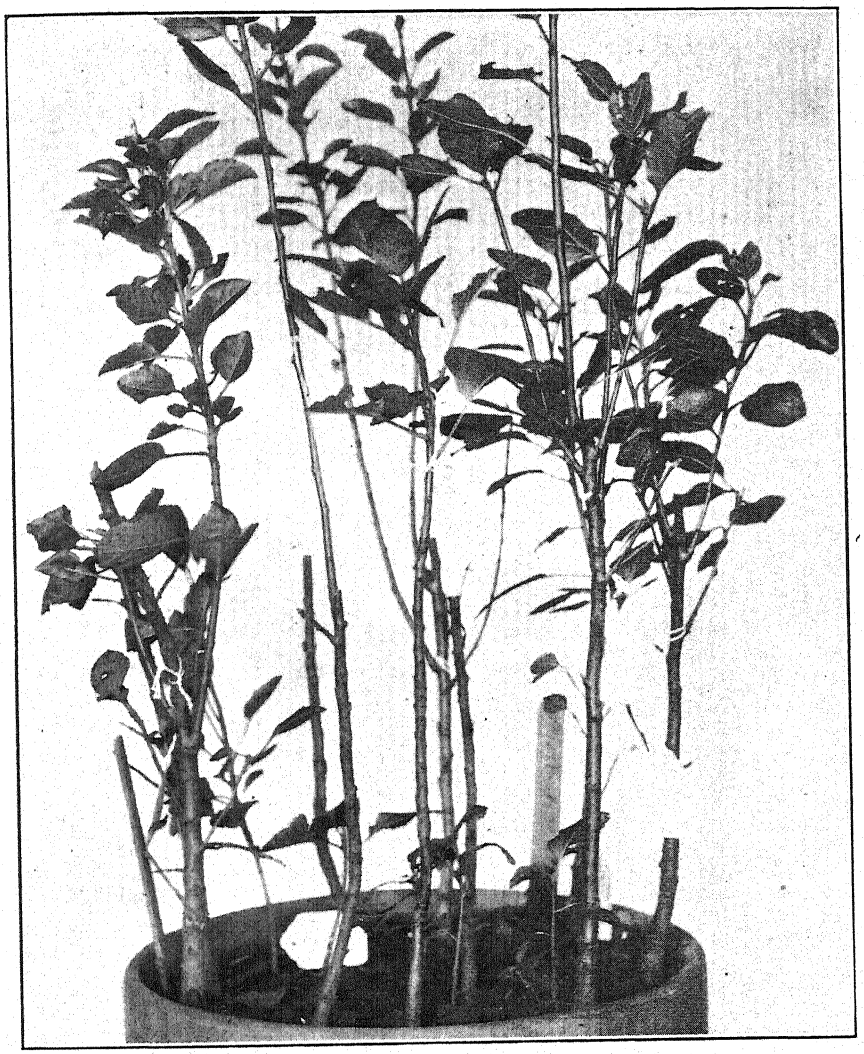


FIG. 1. Cold-storage seedlings, showing most of the 1929 twig and leaf growth above the 1927 trunk cankers. The small seedlings are from Yates seed planted in the pot.

infected, as seen in figure 3, *1*. This difference was chiefly due to the fact that the Yates seedlings being shorter had more leaves below the cankers where spores readily fell upon them. The majority of the leaf spots developed single pycnidia, the characteristic form which the specific name of the fungus is derived.

On August 24 typical petiole cankers (Fig. 2, *2*) were found on several leaves on the small Yates seedlings. These petiole cankers contained

numerous pycnidia. In a single case *Phyllosticta solitaria* had progressed from one of these petiole infections and formed a small but typical canker on the trunk of a Yates seedling. This canker could not be photographed as it grew in the pot, and it was not desirable to sacrifice the seedling for a photographic record at that time.

As there was no other near-by source from which the leaf spot and petiole infections could have developed, their presence on the Yates seedlings indicated that infection came directly from the renewed growth of the blotch fungus from the old cankers on the stored seedlings.

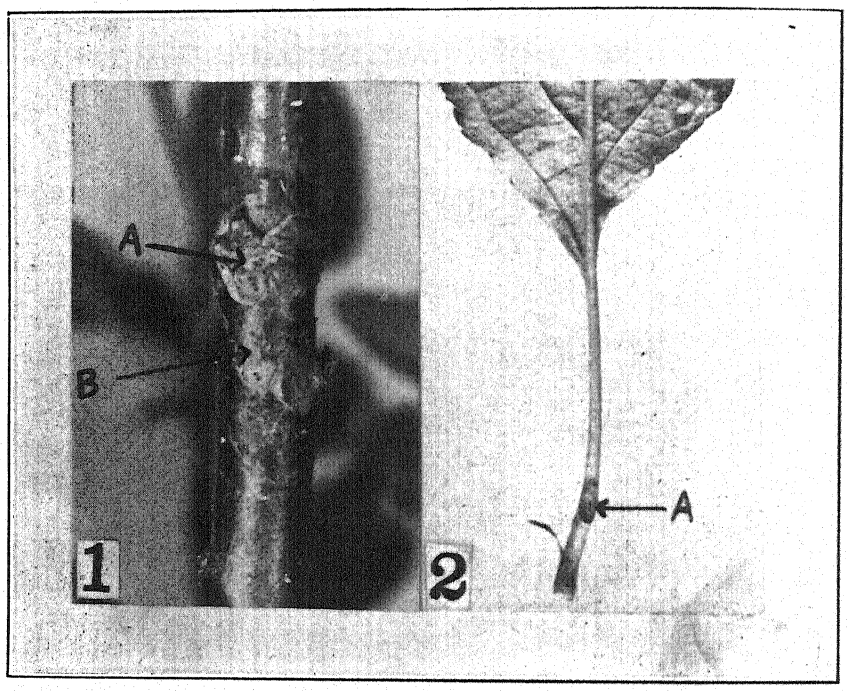


FIG. 2. *1.* An enlargement of a potted cold-storage seedling, showing the 1927 canker at A and at B the new canker tissues produced in 1929 by an outgrowth of the blotch fungus from the 1927 canker.

2. Leaf from Yates seedling growing in pot with the cold-storage seedlings. Note the typical petiole infection of the blotch fungus at A.

Of a total of 83 blotch-fungus cankers on the potted seedlings, six showed no signs of new growth and appeared to be dead, while 77 showed typical new growth, as seen in figure 3, *1*. In nine cases *Phyllosticta solitaria* had grown from old cankers directly into new growth produced by buds adjoining the old cankers, thus enlarging the old cankers on both the 1927 and the 1929 growth. These facts indicate that *P. solitaria* will live for nine months on seedlings held at temperatures of 34° to 36° F.

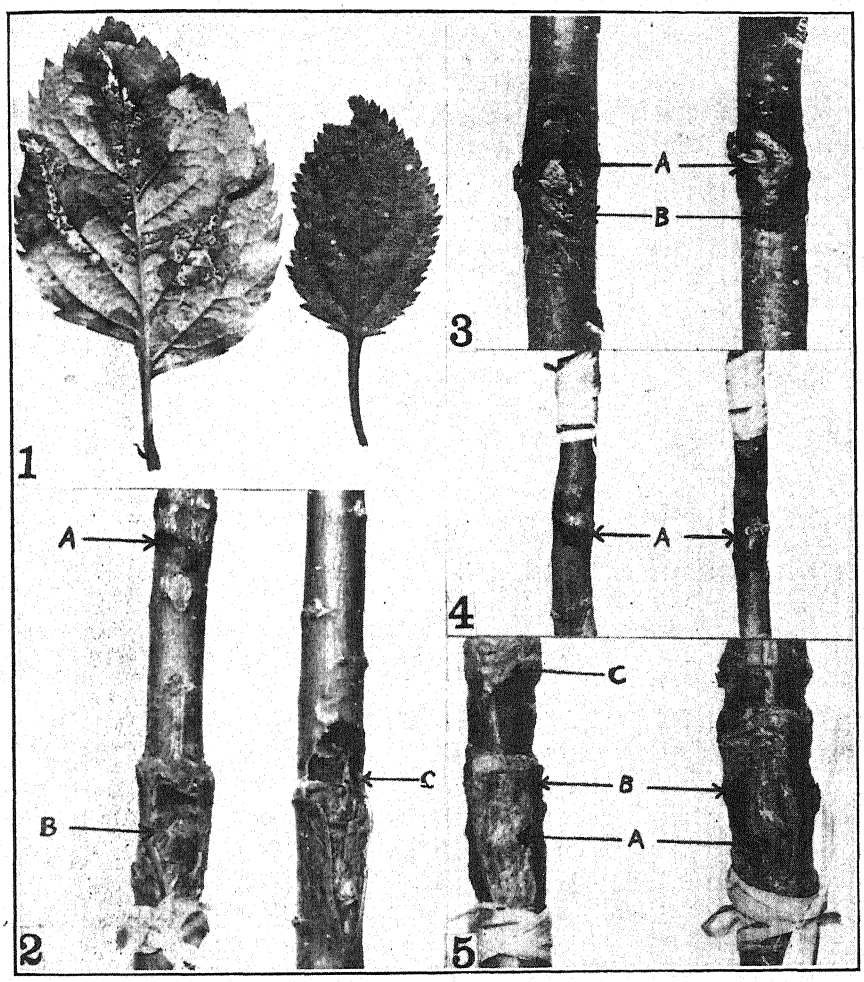


FIG. 3. *1.* Leaves from Yates seedlings growing in pots. Note the circular spots on the upper surfaces of the leaves, with single pycnidia typical of blotch leaf infection.

2. Cold-storage seedlings collected in a commercial nursery September 7, 1929. On the seedling to the left the 1927 blotch canker, with new 1929 growth at A, is well above the inserted bud B and would be removed in cutting the seedling top back to the bud. On the seedling to the right the new blotch canker growth at C adjoins the inserted bud so closely that it could not all be removed in topping.

3. Lined-out apple seedlings taken from the test plat August 26, 1929. Note the 1927 cankers at A and the new 1929 canker growth at B produced by the growth of the fungus from the old cankers.

4. Two cold-storage seedlings collected in a commercial nursery on September 7, showing the new 1929 blotch canker growth at A. These cankers below the buds, which are enclosed in the raffia wraps, would not be removed in topping the seedling stocks.

5. Cold-storage seedlings collected in a commercial nursery September 7, 1929. The vertical and horizontal cuts on the seedling stocks are directly into blotch cankers. The inserted buds at A are almost surrounded by the new 1929 blotch-canker tissue B, which developed from 1927 cankers like C.

DEVELOPMENT OF PHYLLOSTICTA SOLITARIA IN CANKERS ON STORED SEEDLINGS LINED OUT IN THE FIELD

On April 8, 1929, the less heavily infected seedlings from cold storage were lined out in a section of nursery row for comparison with nonstored seedlings as to growth and vigor. While the percentage of loss through subsequent death was higher in the stored seedlings, those that lived made satisfactory growth and were ready to bud as soon as nonstored seedlings of the same grade growing under similar conditions.

On August 26 the lined-out blotched seedlings were examined to determine to what extent the fungus had developed under field conditions. Figure 3, *3* is typical of the new canker development in these seedlings. In all cankers examined, more or less new growth had developed about the old cankers. This indicated that *Phyllosticta solitaria* was alive in nearly all of the cankers at the time the stored seedlings were lined out in the field. These data indicate that blotch-fungus-infected stored seedlings are as dangerous in spreading the causal fungus as are infected seedlings grown the previous season and not held in cold storage.

BLOTCH CANKERS ON STORED SEEDLINGS LINED OUT IN A COMMERCIAL NURSERY

To obtain additional data on the extent to which *Phyllostica solitaria* lives over on cold-storage seedlings, the nursery from which the stored seedlings came was visited on September 7. This nursery, about 200 miles from the University farm, consists of several separated farms. The farm on which these seedlings were set was at least a mile from any apple nursery stock. The particular field had been in a rotation of general farm crops, and no apple trees had been grown there for six years. With such isolation it would be expected that the existing blotch-fungus infection had been introduced on infected seedlings.

As is the practice in Tennessee nurseries, the majority of the seedlings removed from cold storage on February 8 had been used for whip grafts. These grafts, which included a number of commercial varieties, had been lined out according to nursery practice and were making satisfactory growth. As the graft unions in all cases were well below the ground, the seedling stocks could not be examined for blotch cankers. A careful examination of the old and new scion growth aboveground showed no evidence of blotch infection on thousands of whip grafts, many of which are susceptible varieties.

Adjoining the block of whip grafts was a block of 5,000 of the seedlings from cold storage which had been lined out in April, 1929. These seedlings

had made good growth and had been budded during the latter part of July and early August. In nearly all cases the buds were still covered with raffia wraps, but active blotch cankers were readily found on the seedling stocks, as seen in figure 3, *4*. Such cankers on the seedlings lower than the points where buds were inserted might easily be overlooked by budders and probably would not be detected in subsequent field operations. Spores liberated from pycnidia in these cankers would be a ready inoculum for new bud growth the following season. On further examination seedlings like those of figure 3, *2*, were found with active blotch cankers both above and adjoining the inserted buds. The cankers some distance above the buds would probably not be a source of danger because cutting back the tops to the buds the following spring would remove them before new bud growth started. In the case of cankers adjoining the inserted buds, however, the danger of infection would be much greater, since infected tissues would not be entirely removed in cutting the tops back to the buds in the spring. The fungus in that part of the canker left on the seedling stock could attack the bud either by direct mycelial growth as the canker enlarged or by spores from pycnidia attacking the new growth from the inserted bud.

It is not a general practice to give apple nursery stock the frequent and thorough Bordeaux sprays which are effective in controlling blotch of bearing orchard trees. Even such sprays, which would reduce spore infection, would not assure blotch-free trees as long as blotch-fungus cankers adjoined the inserted buds.

Ordinary nursery workers are not familiar with the appearance or seriousness of the blotch fungus on apple seedlings, as is clearly demonstrated by figure 3, *5*. It will be seen that preparatory to inserting the buds, the budder had made both cuts into active blotch cankers on the seedlings. A satisfactory union of the buds with the seedling stocks had occurred in both cases. On September 7, when the seedlings were collected, the dormant buds appeared normal and healthy. There is small chance of such buds growing into healthy trees, for practically none of the canker tissues would be removed in topping the seedling trees back to the buds in the spring.

The seedlings described and photographed are typical of numerous budded stocks observed scattered over this block of 5,000 lined-out seedlings. In all cases the causal fungus was observed to have grown from the 1927 cankers during the summer of 1929. This indicated that cold storage for a period of nine months does not materially reduce the danger of blotch infection from cankered seedlings.

This ability of *Phyllosticta solitaria* to live in an apparently dormant condition from November or December, 1927, to March or April, 1929, and then renew active growth and spore production indicates the importance of inspecting and culling out blotch-cankered apple seedlings intended for holding in cold storage, the same as advised for seedlings intended for propagation purposes the following year.

SUMMARY

Since 1923 blotch cankers have been found on apple seedlings shipped into Tennessee from midwestern nurseries.

Blotch cankers were found early in March, 1929, on apple seedlings grown in midwestern nurseries in 1927 and held in cold storage throughout the growing season of 1928. There was no evidence that *Phyllosticta solitaria* made any growth during the storage period.

Stored seedlings having numerous blotch cankers, set in large pots in the greenhouse, showed new canker-fungus growth as the season advanced.

Leaves on the stored seedlings and on small seedlings grown directly from seed planted in the pots developed typical blotch leaf spots.

Later in the season typical blotch petiole infections developed on the trees from seed planted in the pots.

Similar cold-storage seedlings in field test blocks developed new canker growth in 1929, enlarging the 1927 cankers.

Trees from the same lot of stored seedlings planted in a commercial nursery about 200 miles from the University farm showed similar new canker development in 1929 from the 1927 cankers.

Observations on the location of inserted buds in relation to blotch cankers indicated that a large percentage of infection results.

The renewed activity of *Phyllosticta solitaria* in 1929, after remaining dormant since the fall of 1927, stresses the importance of inspection and removal of all blotch-infected seedlings from stocks intended for holding in cold storage.

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UNIVERSITY OF TENNESSEE,
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REPORT OF THE FOURTEENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meetings of the Pacific Division of the American Phytopathological Society were held in conjunction with those of the Pacific Division of the American Association for the Advancement of Science and Affiliated Societies, June 19–20, 1930, at the University of Oregon at Eugene.

At the business meeting, which preceded the presentation of papers, the following officers were elected to serve the society for the next two years:

President .....E. CARSNER, U. C. Citrus Experiment Station, Riverside, California.
Vice-President .....J. M. RAEDER, University of Idaho, Moscow, Idaho.
Secretary-Treasurer .....B. A. RUDOLPH, U. C. Deciduous Fruit Station, San Jose, California.
Councillor .....C. E. OWENS, State College, Corvallis, Oregon.

The attendance of members at the meetings was not so large as hoped for, but those present were enthusiastic and deeply appreciative of the many courtesies extended by their colleagues in the Pacific Northwest. After adjournment the visiting members were taken in automobiles to the State College at Corvallis, a distance of about forty miles. Here an opportunity was afforded to inspect the laboratories and experimental grounds of the Department of Plant Pathology. In the evening a delicious supper was served on the lawn, after which the visiting members motored back to headquarters at Eugene.

There was an average attendance of nineteen members with a varying number of visitors. Twenty-one papers were presented, abstracts of which follow.

B. A. RUDOLPH, *Secretary-Treasurer*

ABSTRACTS

Some inoculations of Juglans with Phytophthora-like fungi.—C. O. SMITH and J. T. BARRETT.

With the use of the California black walnuts as a stock for the English walnut, has appeared the disease known as crown rot. This is an infection of the black walnut crown. Either of two fungi may be the causal organism (1) a species of Phytophthora of the *Phytophthora cactorum* type or (2) a Phytophthora of the Pythiacystis type. The disease may spread to the English walnut trunk and extend upon it for several feet. The walnut Phytophthora has been observed by Barrett producing vegetative growth and oospores on crown-rot bark of English walnut that was wrapped for five days in paraffin paper.

Artificial inoculations on *Juglans californica* (the southern California black walnut) has shown it to be most susceptible. Two hundred fifty-three inoculations gave 80 per cent positive lesions. *Juglans hindsii* (the northern California black walnut), 350 inoculations, gave 50 per cent positive infection. *Juglans regia* (English walnut), 209 inoculations, showed 82 per cent positive infection. The susceptibility of *J. regia* to artificial inoculations, is in marked contrast to its apparent resistance to the crown-rot disease as shown by field observations. The lesions on the English walnut are much smaller than those on *Juglans californica* where, on some mature trees, they reached the size of 60 and 90 inches in a year's time. *Juglans sieboldiana, Juglans nigra, Juglans major* and a Juglans from Mexico (*J. pyriformis*) as well as almonds, peaches, sweet cherry, pear, *Prunus domestica,* and lemon fruits are all susceptible when inoculated. Cultures of *Phytophthora cactorum* from various sources and hosts when inoculated into *Juglans californica* produced lesions typical of walnut crown rot.

It has been difficult to isolate the pathogenes from the diseased crown of *Juglans californica* because of a toxic exudate from the cut tissue. The isolations are more successfully made from the infected English walnut tissue.

A test was made of the toxic effect of one gram of walnut bark boiled in 15 cc. of glucose-potato agar, then plated and inoculated with mycelium of the two walnut pathogenes. The following is the measure of mycelial growth after 20 days in petri dishes:

Diseased southern California black bark and agar	1 mm.
Normal " " " " " "	1.5 cm.
Diseased English bark and agar	6.0 cm.
Normal " " " "	2.25 cm.
Control agar plates covered with growth.	

Relation of cane blight (Leptosphaeria coniothyrium) to lesions of yellow rust (Phragmidium imitans) of red raspberry in Oregon.—S. M. ZELLER.

In a recent article (Jour. Agr. Research 34: 857–863, *illus.* 1927) the writer described the yellow rust of raspberry and pointed out the serious cankerous effects of cane lesions near the ground. Since then much greater losses of canes have been found to be produced by a subsequent infection of these rust lesions by *Leptosphaeria coniothyrium.* The cane blight causes long basal cankers. This combination of the two diseases has caused a loss of 50 to 60 per cent of the canes in the most severe cases, and an estimated annual loss in crop of $75,000 in the Willamette Valley in 1928 and 1929. The control measures suggested are complete sanitation to control basal infections of the canes by the rust. That is, old stubs should be removed and old canes cut so as to leave no stubs, then late fall plowing toward the rows after all the leaves are down.

A blue mold affecting tulips.—F. D. HEALD and KENNETH BAKER.

This trouble was found on one large shipment of bulbs, 60 per cent of them showing lesions involving several of the outer scales, with a Penicillium fruiting abundantly. The relation of the fungus to the rotting of bulbs was determined by pure-culture inoculations on bulbs held in moist chambers and on others planted in the greenhouse. At the end of three weeks the bulbs in the greenhouse showed 40 per cent completely rotted, while the others showed lesions 15–25 mm. in diameter. Those in the moist chambers showed lesions of similar size but no complete rotting. The blue mold continued to spread, involving the entire surface of the bulbs with variable penetration. Inoculations into incisions grew more rapidly, but infections were also obtained by dipping bulbs in a suspension of the blue mold spores, the number of lesions varying from one to six per bulb.

Inoculations of the tulip blue mold in comparison with *P. expansum* were made on tulips, gladioli, and apples. The tulip mold was able to rot apples but not gladiolus bulbs. The rot on apples was much slower than that caused by *P. expansum* and the decayed tissue darker and firmer. When Jonathan apples inoculated with *P. expansum* were completely rotted, the parallel inoculations with the tulip mold showed lesions 3.5–4.5 cm. in diameter.

A witches' broom of ocean spray (Holodiscus discolor Max.).—S. M. ZELLER.

Since 1925 observations of a severe brooming and dwarfing of *Holodiscus discolor* Max. have been made. This disease has been found in the foothills of the western slope of the Cascade Mountains from Linn to Clackamas counties, Oregon. The internodes of the stems are short and stouter than normal. The stems have little tendency to branch so there are usually no blossoms. There is a multiplication of buds at each node. These produce very spindly short laterals. Leaves are small and turn a bronzy red early in the summer. Budding of diseased nodes into healthy stems and the transfer of aphis from diseased to healthy plants have both apparently induced the disease.

Budded branches show the symptoms first and then there is a spread of abnormal growth to other parts of the plant. Insects which might induce such abnormal bud production have not been observed. The symptoms and performance thus far observed would indicate this to be a virus disease.

The effect and the rate of spread of "streak" on greenhouse tomatoes.—LEON K. JONES.

"Streak" of tomato produced by combined viruses of potato and tobacco reduced yields of tomato 50 per cent in an experimental greenhouse planting. Within sixty days after four plants were inoculated in a bed, the remaining sixty-one plants were affected with streak. This spread of the disease was due to cultural and pruning practices, as the plants were free from aphids. Hands were carefully washed with soap and water before pruning in two adjacent control beds of sixty-five plants each. These adjacent beds remained free from streak through the sixty-day period mentioned above. This experiment shows that streak may be spread very rapidly by ordinary pruning methods and that frequent washing of hands with soap and water while pruning reduces the rate of spread of this disease.

Studies of perennial-canker disease of the apple.—J. S. COOLEY and P. W. MILLER.

Successful inoculations of *Gloeosporium perennans* were accomplished throughout the rainy season of 1929 and 1930 or from September to June. The highest percentage of positive infection was in the fall. A study was reported of the susceptibility of wounds made by breaking spurs, by hacking with a saw, by making a smooth incision with a knife, by brushing with a pointed-head mallet, and by driving a small nail into the wood. Those wounds made with a nail and by bruising gave much higher percentages of infection than those made with a sharp tool.

Infection in the inoculation experiments reported was accomplished in fresh wounds that were free from woolly-aphis infestation.

Experiments dealing with the mode of infection under natural conditions of healing calluses was reported. Infection may occur on fresh wounds, but much of it occurs at healing calluses that have been injured by cold. In the summer of 1929 the canker fungus was isolated from pruning-wound calluses having one or more dead areas on the margin that resembled winter injury followed by canker infection. The dead portion of the calluses varied from two to ten or more millimeters in width. In March, following the cold weather in January, 1930, isolations were made from calluses free from

canker in 1929 but showing dead margins that apparently had resulted from recent winter injury. Most of these first attempts at isolating the fungus gave negative results, but the later isolations showed an increasing number of positive infections by the canker fungus. This indicates that the margins of healthy calluses may be injured by cold and that canker infection readily takes place in the winter-injured margins of pruning-wound calluses.

Chemically-treated wraps for the control of Botrytis rot of stored fruits.—J. S. COOLEY.

A rot very prevalent in stored pears and sometimes in apples is caused by Botrytis. The greater part of the loss resulting from this rot is caused by its spread from one fruit to another. The fungus grows through the paper and attacks noninjured fruits The data presented show the efficacy of a chemically-treated wrapper in preventing the spread of this rot.

Pears were wrapped and stored in wraps treated with salts of copper, iron, chromium, nickel, and sodium and also nontreated paper. One layer in each box was inoculated with Botrytis and all fruit was wrapped in treated paper of a given kind. After four or five months of storage, the lot in nontreated wrappers contained only one or two sound fruits to the box while the fruit in the treated wrappers showed that the spread of the fungus was materially affected by the toxic salt in the paper.

A very successful wrapper was prepared by wetting with a 2½% solution of hydrated copper sulphate, the paper absorbing 5.6% by weight of copper sulphate, or 1.4% metallic copper. When the fruit was wrapped in such papers the spread of the Botrytis rot was controlled without chemical injury to the fruit.

The avocado disease called sun-blotch.—WM. T. HORNE and E. R. PARKER.

THE name sun-blotch[1] has been given to a peculiar avocado disease characterized by obscure longitudinal streaks or blotches in the fruit, streaks in vigorous shoots, roughening and decumbency in older stems, and rough bark of trunks. An irregular leaf variegation observed by the writers is believed to be connected with the disease. In green fruit the streaks remain light or yellowish, but in the varieties they become variously red-purple. In its more intense phase the disease is very serious, but symptoms may be slight.

Sun-blotch scions produce sun-blotch trees when set in healthy stocks. Healthy scions in affected stocks probably also produce diseased trees.

In three cases, where diseased scions were set in healthy stocks by the junior author, shoots subsequently arising from the stocks were clearly affected with sun-blotch. Various inoculations and treatment with X-rays have thus far failed to produce the disease.

Apparently sun-blotch is an infectious chlorosis.

A disease of date palm inflorescences.—L. J. KLOTZ and E. C. RABY.

It seems opportune to report at this meeting the recent finding in the Coachella Valley of a disease of date palm inflorescences which may become of serious economic importance. A hasty survey revealed but one infected palm. The disease apparently limits its attack to the fruit stalk and rachises of the young inflorescences. The latter were found diseased even before the spathe had ruptured. On the spathe, circular to elongated lesions, sorghum brown (Ridgway) on the exterior surface and ranging from this color to a mahogany red or bay on the interior surface, marked the starting points

[1] Coit, J. E., Sun-blotch of the avocado, a serious physiological disease. Yearbook Calif. Avocado Ass'n., 1928, pp. 27–29, 5 illus. Los Angeles, Calif., Sept., 1928.

of the disease. On removal of a portion of the infected spathe it was found that the fruit stalk bore depressed, brown (warm blackish) to black necrotic areas, circular to oblong in outline. The twisted deformed rachises were completely involved, blackish brown to black, and devoid of flowers. Microscopic examination showed them covered by dark brown, unicellular, oval conidia. The rachises of fruit bunches attacked later in their development had blackened, depressed lesions similar to those on the fruit stalk, and some were completely severed by the decay. The affected tissue was in all instances dry and firm, and each area bore the black powdery spores. A gray covering on some of the lesions was found due to conidia of *Fusarium* spp.

While the malady has features in common with the Khamedj disease of date palms in Northern Africa, described by Cavara, Chabrolin, and others, the black lesions do not have the white, tomentose covering of the fungus, *Mauginiella scattae* Cav., to which they ascribe the cause of Khamedj. From the interior tissue of the material collected at Indio, a Torulae-like Hyphomycete, *Thielaviopsis* sp., was invariably isolated. On culture media this organism produces a white, aerial growth bearing chains of hyaline conidia which rapidly darken as they mature. Sufficient time has not elapsed since our inoculations to permit us to report on the pathogenicity of this fungus. It may be mentioned that the senior author has found this organism also in connection with the bud scorch and "fool" diseases of date palm.

A graft-infectious disease of the cherry.—T. E. RAWLINS and W. T. HORNE.

A severe disease of the sweet cherry occurs in several valleys in California. Diseased trees produce fruit that is more or less conical in form. The development of such fruit ceases just before ripening, and the fruit hangs on the tree in this immature state for some time, finally shriveling without ripening. During early autumn the leaves on diseased trees may show a peculiar reddish purple coloration along the base of the midrib which extends out along the larger veins. This leaf symptom varies considerably according to the season and may be difficult to detect during certain seasons.

The disease has been transmitted by grafting scions from diseased Napoleon trees into healthy trees.

Trees on Mahaleb roots have shown considerable resistance to the disease, while those on Mazzard roots are very susceptible.

Leaf roll transmission from potato to other solanaceous plants by means of Myzus persicae.—T. P. DYKSTRA.

Leaf roll of potato was transmitted by means of *Myzus persicae* to tomato, pepper, *Datura stramonium, Datura tatula, Solanum nigrum,* and *Solanum dulcamara.*

Infections varying from 70 to 100 per cent were generally secured. After symptoms became apparent in the plants, aphids were colonized on them, and, after a few days, transferred to potato. In every case a high percentage of leaf roll developed on potato when return inoculations were made from infected plants.

Cross inoculations from Jimson weed to tomato, from tomato to Jimson weed, from *Solanum dulcamara* to tomato, from tomato to tomato, and from Jimson weed to *Solanum nigrum* were also made, and resulted in high percentages of infection.

Symptoms of the two Datura species and of *Solanum nigrum* are characterized by chlorosis and rolling and subsequent rigidity of the leaves.

Leaves of infected tomato plants become stiff and leathery, but this is the only distinguishing symptom, and the disease on this host is somewhat difficult to detect. Pepper plants do not show any marked symptoms until after the disease has been present for several weeks; then the leaves begin to roll and become leathery.

Some experiments in fresh fruit disinfection.—H. P. BARSS.

Four years' tests with disinfectants on fresh prunes and cherries for the suppression of decay during storage or shipping were described. Formaldehyde, used as a dip or three-to-six-minute soak, in strengths of one to one hundred or greater, was found to give marked protection, although the higher concentrations sometimes resulted in skin injury and affected the flavor. Diatomaceous earth, impregnated with four to five per cent of formaldehyde and dusted over the fruit, gave excellent control but affected the skin somewhat. The most practical protection was obtained through the use of a sheet of absorbent paper impregnated with formaldehyde placed in the bottom of the box of fruit and another in the top. When the dose was not too strong, decay was suppressed more effectively than by the liquid treatments and without perceptible injury to flavor, color, or luster. Thus far the latter method has not been tested on prunes. Borax alone and with boric acid in solution, acetic acid, hexylresorcinol, and a commercial preparation yielding free chlorine proved inferior or useless. In general, washing often reduced the effectiveness of liquid treatments.

Didymosphaeria oregonensis, *a new canker organism on alder.*—L. N. GOODDING.

This paper describes as new a species of Didymosphaeria found associated with a canker on living alder in the Pacific Northwest. The name **Didymosphaeria oregonensis** Goodding is proposed. The distribution of the new species is given as western Oregon and Washington. There also is included a brief consideration of its taxonomic relationship and economic importance.

Cladosporium species from apple fruit and the perfect stage of Cladosporium herbarum Lk.—GEORGE D. RUEHLE.

In connection with a study of the fungi causing decay of apples in cold storage, three species of Cladosporium were found to be weak parasites of the apple.

The first produces rapidly growing fluffy colonies and light brown cylindric conidia in long chains. It is considered to be a new species.

The second produces dense slow growing colonies and conidia typical of the form species, *C. herbarum*. When grown on mature sterilized wheat leaves for six months at 10° C., this form produced large numbers of black fertile perithecia of the Mycosphaerella type. On germination, single ascospores produced colonies of the *C. herbarum* type. The ascigerous stage is believed to be identical with *Mycosphaerella tulasnei* described in 1893 by Janczewski as the perfect stage of *C. herbarum*. Positive confirmation of Janczewski's report has been lacking until now.

The third form was identified as *Homodendron cladosporioides* Sacc. and produces no perithecia.

As a group these forms are of little importance on stored apples, although of frequent occurrence on this host. They produce small, shallow dark rots around stem punctures and worm holes but, otherwise, do little damage.

In contrast with seedling stock, apparently healthy potato tubers are virus carriers.—GROVER BURNETT and LEON K. JONES.

Tomato plants were inoculated with macerated leaf tissue of commercial potato-tuber stock and with macerated leaf tissue of potato seedlings, each combined with macerated leaf tissue of tobacco plants affected with common tobacco mosaic.

Plants produced from eighty-eight apparently healthy commercial tubers and thirty-two known to be virus-infected, representing eight commercial varieties, were used in the tests. Each virus-infected tuber showed symptoms of one of the following virus dis-

eases: crinkle, crinkle mosaic, leaf roll, mild mosaic, rugose mosaic, unmottled curly dwarf, witches' broom, super-mild mosaic, and spindle tuber.

"Streak" of tomato developed in every case when macerated tissue from these plants was mixed with macerated tissue of tobacco plants affected with common tobacco mosaic and this juice rubbed onto healthy tomato plants.

In no case did streak develop on tomato plants inoculated with macerated potato-seedling tissue combined with macerated mosaic-tobacco tissue. Forty-seven potato seedlings were used in inoculating 245 tomato plants. Series of control plants were alternated with series of inoculated plants. The 438 plants used as controls remained healthy during the tests.

These results tend to show that potato seedlings do not carry this virus which may be present in all commercial tuber stock.

European and American brown-rot blossom blight in western Oregon.—H. P. BARSS.

Serious blossom blight in cherries occurred in many orchards in the Willamette Valley in 1929 and a considerable amount appeared again in 1930. Isolations from material obtained from widely scattered orchards showed that, while both forms of brown rot, prevalent on the Pacific Coast, were present in the blighted blossoms, the European form, *Sclerotinia cinerea* (Bon.) Schröt. forma *pruni* Wormald, predominated by three to one in 1929 over the common American form, *Sclerotinia fruiticola* (Wint.) Rehm (= *Sclerotinia americana* Nort. et Ezek.), and in 1930 greatly exceeded the latter. From green cherries and prunes, attacked shortly after the shucks fell, only the American form was isolated in the same year. Attack by the European brown rot was reported on sweet-cherry foliage, flowering-almond twigs and cherry leaves previously invaded by the witches' broom, Exoascus.

Measuring duration of effectiveness of Bordeaux sprays to perennial canker spores.—J. H. CRENSHAW.

A method is described for determining how long Bordeaux sprays remain effective in preventing germination of perennial canker spores under ordinary orchard conditions.

Trees were sprayed at intervals throughout the rainy season. After exposure to weathering for different periods, twigs were removed for study. These were inoculated with spore suspensions and the spores given an opportunity to germinate on the twigs. They were then transferred to glass slides for microscopic examination.

Some preliminary measurements were made. They indicate that sprays applied late in December were effective in March, but that sprays applied as late in the fall as October 29th were ineffective before February 22nd of the following year.

Electrophoresis of tobacco-mosaic virus.—WILLIAM N. TAKAHASHI and T. E. RAWLINS.

Tobacco-mosaic virus was subjected to electrophoresis at various pH values. The virus moves toward the anode at pH 4 to 9. No movement was detected between pH 1.2 and 3. The electrophoretic behavior of the virus was similar to that observed for bacteria by other workers.

Some pathological changes induced in citrus by a deficiency of boron.—A. R. C. HAAS and L. J. KLOTZ.

In sand and water cultures lacking boron, several species of Citrus showed the following symptoms of decline: Leaves curled along the midrib with the tip of the leaf bending downward and inward; leaves brownish or yellowish green, often with a yel-

lowing along the midrib; midrib or veins conspicuous, corky and split; and a progressive loss of affected leaves. In severe cases there is a tendency toward "multiple bud" formation due to incipient shoots dying when barely visible. This and other symptoms are similar to those found in exanthema. When the bark of the internodes of the twigs, or in severe cases that of the trunk, splits, an amber-color gum oozes out. Eventually the cracks may widen so that the woody tissue is exposed. In severe cases the apical portion of the branch dies back. The roots become dark brown, fail to elongate, and in advanced cases the rootlets decay. On the addition of a suitable concentration of boron to the culture solution the symptoms of decline disappear and normal growth resumes.

Anatomical studies show that boron is essential for cell division in the meristematic tissue of the growing points, such as buds, and it is likewise essential for cambial activity.

The abnormal accumulation of carbohydrates in the leaves of boron-deficient Citrus, coupled with the fact that the phloem tissues are destroyed, would show that translocation is seriously interfered with. Reduction in the total sugar content of the leaves accompanies recovery of the tissues brought about by the addition of boron to the culture solution.

Some experiments with crown-gall bacteria.—F. A. PATTY.

When plants of tobacco, castor bean, geranium, and garden bean were sprayed with suspensions of crown-gall bacteria, the organisms entered the leaves through natural openings, as indicated by the recovery of virulent organisms from the mesophyll of the leaves after stripping away the epidermis. Except for the consistent recovery by culture of the crown-gall organism from within sprayed leaves no other indication of the presence of the bacteria appeared, for there was no gall formation or other indication of disturbance in the development of the leaf tissues, even when partially developed leaves were inoculated by this method and held under observation for four weeks. Puncture inoculations with the same strains of the crown-gall organism produced visible enlargements on the stems within six days.

On the control of bacterial blight of walnuts.—B. A. RUDOLPH.

Bacterial blight is by far the worst disease with which walnut growers must contend. During the past three years extensive spraying experiments have been conducted. Virtually all of the better-known chemicals and bactericides have been employed, many of which are harmless to other plants but very injurious to the walnut. Three copper-containing compounds, Bordeaux mixture, basic copper acetate, and ammoniacal copper carbonate, have proved effective in the control of the disease and, incidentally, occasioned distinctly less injury to the trees than most other materials. Bordeaux 8–4–50 (the lime used reduced to one-half) has given the best results of all. The strategic period for securing maximum results with one application of spray is when the catkins are fully elongated and about to shed pollen. The increase in crop on trees sprayed at this time has more than justified the expense of spraying in many instances. In badly diseased orchards or in orchards situated in localities where climatic conditions favor ready infection and the rapid spread of the disease, multiple spraying must be employed. The first spray is given as before, and subsequent sprays follow several months apart to protect the growing nuts which are susceptible at any time until the harvest in October. Thoroughness of application of the spray cannot be stressed too much.

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THE RELATION OF THE CABBAGE MAGGOT AND OTHER INSECTS TO THE SPREAD AND DEVELOPMENT OF SOFT ROT OF CRUCIFERAE[1]

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INTRODUCTION

In 1927 Leach (11) showed that the seed-corn maggot (*Hylemyia cilicrura* Rond.) is a common agent of dissemination and inoculation of the potato blackleg pathogene. In that paper he briefly called attention to a similar relationship between the cabbage maggot and the soft rot of cabbage. This paper is an account of a study of the bacterial soft rot of cabbage with special reference to the rôle of the cabbage maggot (*Hylemyia brassicae* Bouché) in the dissemination of the pathogene and the development of the disease. Since the completion of this work Bonde (2), in a preliminary note, also has reported the cabbage maggot as a disseminating agent of bacterial soft rot of Cruciferae.

THE SOFT ROT OF CABBAGE

Jones (10), in 1900, proved that the soft rot of cabbage and of many other vegetables is caused by a bacterium which he named *Bacillus carotovorus*. Harding and Morse (9) later showed that the disease can be caused by a group of closely related bacteria, considered by them to be variant strains of the single species, *B. carotovorus* Jones.

Although Jones (10) proved that *B. carotovorus* was a wound parasite and that it could penetrate the unbroken epidermis, the manner in which it is disseminated and inoculated into plants never has been explained satisfactorily. Insects have frequently been mentioned as agents of dissemination. Chupp (5) in his general discussion of soft rot of vegetables makes the following statement: "The organism, *B. carotovorus,* is common on so many hosts both in storage and in the field, that it lacks no opportunity of

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[2] The writer wishes to express her appreciation to Dr. J. G. Leach, under whose direction the work was carried on, to Dr. E. C. Stakman for valuable assistance, and to Dr. R. U. Cotter for specimens of the onion maggot. She is indebted also to Dr. C. E. Skinner of the Department of Bacteriology for criticizing the manuscript.

becoming established in new crops in summer. Carried by insects or in any other manner in which microscopic bodies may be disseminated, the bacteria gain an entrance into the injured tissue, where they soon dissolve the middle lamella, leaving only a slimy mass of free cells;'' also, ''insects and cultivating tools carry the organism in the field.'' In 1926 Leach (12) showed that the dipterous insects, *Elachiptera costata* Leow. and *Scaptomyza graminum* Fall., are capable of disseminating the pathogene and of inoculating it into celery, where it produces a heart rot.

Symptoms.—Soft rot of cabbage may become manifest in several ways. In the field it is most destructive as the so-called ''stump rot.'' Stump rot appears most frequently rather late in the season when the plants approach maturity. The first symptoms usually consist of a wilting of a few of the outer leaves and a cessation of growth. Frequently the head may appear normal when viewed casually. However, upon careful examination, it usually will be found that the center of the head is a mass of decaying tissue, extending downward into the pith of the stem. In advanced stages such heads may be pulled from the stem easily. Observations throughout the season show that the decay arises first in or on the base of the older leaves, whence it spreads upward into the center of the head. After the decay has reached the head, it is only a matter of time until the entire head is reduced to a mass of rotting tissue, leaving the stem as a ragged stump with the core completely destroyed.

Soft rot is primarily a disease of the succulent tissues of the plant; the woody portions are not affected. On young plants it is commonly confined to the tissues close to the injuries caused by the cabbage maggot, as will be pointed out later. Soft rot is very destructive to cabbage heads in storage or in transit, especially if the disease has been common in the field, and if the cabbage is stored in poorly ventilated places at relatively high temperature.

THE CABBAGE MAGGOT

The cabbage maggot (*Hylemyia brassicae*) is a common and well-known insect. It has been described so adequately by Schoene (15), Gibson and Treherne (6), and other entomologists that only a brief description of it will be given here. It is distributed throughout the United States, Canada, and Europe. It attacks all members of the Cruciferae, being especially destructive to cabbage, cauliflower, and radish. The first brood of the adult fly emerges in the spring and early summer. It resembles the house fly, but is smaller, being only about one-fourth of an inch long, with a narrower body and proportionately larger wings. It is grayish, with three dark stripes on the thorax and one along the abdomen, and has many stiff hairs on the body. The male is slightly smaller than the female. Morpho-

logically there is no difference between the adult female fly of the cabbage maggot and that of the seed-corn maggot, but the male adult fly of the cabbage maggot can be distinguished from that of the seed-corn maggot by the presence of a tuft of bristles on the base of the hind femur.

The first brood of flies deposits eggs near the base of the stem of the young host plants. The eggs hatch after a few days. The maggot is white or yellowish and tapers slightly toward the head. When full-grown it is about one-third of an inch long. It has a pair of strong, dark colored, hook-like, rasping, downward-projecting jaws. The newly hatched maggot begins at once to rasp the surface of the young roots, gradually mining into them. It finally ceases feeding, and the skin hardens, forming the puparium, within which the larva transforms to a pupa. Later the adult fly emerges from the puparium.

The attacks of the maggot kill or severely injure the young plants. Although soft rot is frequently present on such plants, it is usually obscured by the mechanical injury caused by the larvae. Because of the relatively large proportion of lignified tissue in the stems of young plants, the rot seldom spreads far beyond the tissues injured by the maggots.

The later broods often deposit eggs at the base of the leaves of the older plants. When these hatch, the larvae burrow into the leaf base and often into the head of the cabbage plant. These tissues are very succulent and especially susceptible to soft rot; following such maggot injury, they frequently decay, giving rise to the condition known as stump rot. In the fall the maggots and the puparia are often found wedged between the leaves of the head, the flies of the last brood frequently laying the eggs on or between the leaves of the well-developed heads. The insect overwinters in the puparial stage in the soil or in the refuse from cruciferous plants. In mild climate it may also overwinter in the adult stage.

Entomologists have observed for many years that laceration of the cabbage-plant tissue by the cabbage maggot often is followed by decay. According to Slingerland (16), Ormerud stated in 1892 that "the maggots bore into the roots and cause mischief, sometimes to a serious extent by the decay so originated." Slingerland expresses entire agreement with this view. Schoene (15) says that "the lacerations of the roots of the cabbage and cauliflower are often accompanied by a decay of the injured part," and "the newly made tunnels are clear, but later they become brown and discolored due to the decay of the exposed tissues."

THE PRESENCE OF THE SOFT-ROT BACTERIA IN THE VARIOUS STAGES OF THE INSECT

The Puparium.—The first collection of puparia for the study presented in this paper was made in August, 1927, from the second brood of maggots, the puparia being collected from soil around both cabbage and radish

plants. The methods used in the isolation of bacteria from the puparia were, in general, similar to those used by Bacot (1) and by Leach (11).

Young puparia, recognizable by the light brown color, were selected for immediate work, the darker ones, evidently a more advanced stage of the insect, being reserved for the study of the adult fly. The puparia were washed carefully to remove all adhering particles of dirt. Next they were immersed for 5 minutes in 5 per cent alcohol. They were then transferred with a flamed needle to a solution of mercuric chloride (1–1000), where they were kept 3 minutes, after which they were removed aseptically to 50 per cent alcohol and kept there for about a minute, being finally placed in tubes of sterile broth to wash off all trace of the disinfectant. Later they were transferred to slants of sterile agar and allowed to remain for several hours. These slants were kept under observation for bacterial growth, since any growth here would indicate the survival of external contamination of the puparia. The puparia were again washed in sterile broth and transferred to fresh agar slants. Both the broth and the agar slants were saved for further observation for bacterial growth.

Seventy-two of 214 puparia so treated produced no growth in the broth or on the agar slants and were kept to await the emergence of the adult fly. Many of the remainder produced no contamination in the broth, but eventually bacterial growth was observed upon the agar slants. This might indicate that the puparia had been sterile externally but that bacteria had seeped out through the air pores. This growth often produced a soft rot when inoculated into cabbage leaves, but no conclusions were drawn from such inoculations.

Various methods of sterilizing the surface of cabbage leaves were tried. Five per cent Formalin gave good results with potatoes and carrots but was not successful with cabbage leaves. Immersing the leaves in alcohol, then burning off the alcohol, was tried. But this often produced a softening of the tissues. Lugol's iodine solution left fumes which apparently interfered with the development of the inoculum. Mercuric chloride solution (1–1000) was fairly efficient, but the best results were obtained with Semesan and other organic mercury compounds in the usual concentrations. The disinfectants were washed off with sterile water, then the leaf pieces were transferred aseptically to sterilized petri dishes containing several layers of moist filter paper. To accomplish sterilization of the dishes it was necessary to subject them to steam at 15 pounds pressure for at least 30 minutes. Cabbage-leaf sections, so prepared, were incubated at 22° to 25° C. for 48 hours and all discarded which showed any softening of the tissue. At least 4 plates were used as checks. If any softening of the tissue took place in any of the checks, the experiment was repeated.

Various methods of preparing the inoculum were tried. Water suspensions of the organism were found more satisfactory than broth cultures, since saprophytes were not always destroyed, even with the most rigid surface sterilization of the cabbage leaves. Such saprophytes develop readily in broth, and, if a softening of the tissues should occur as a result of high temperature and humidity, erroneous conclusions might easily follow.

From day to day, according to the age of the puparia, adult flies emerged from the puparia previously planted on the sterile agar slants until, in all, 53 flies had been so obtained. Many dragged their wings upon the agar and also left foot tracks. After about 24 hours these marks were clearly defined by bacterial growth. The growth from each of these slants was plated on nutrient agar. Cultures corresponding macroscopically, microscopically, and physiologically to *B. carotovorus* were obtained from 27 of the puparia. These were later found to be pathogenic to cabbage, as will be discussed later. Since many of the cultures so isolated were found to consist of saprophytes, it is evident that colonies of *B. carotovorus* are not readily distinguished from many other bacteria present in the puparia. In order to determine if the soft-rot bacteria might be present on the plates and be missed in the selection of colonies, the remainder of the growth on each plate was washed off with sterile tap water and this bacterial suspension inoculated into cabbage-leaf sections. Washings from 11 plates from which no colonies of *B. carotovorus* had been picked produced soft rot. Thus from 53 flies that had emerged from the puparia upon the agar slants, 38 harbored the soft-rot bacteria in the puparial stage.

A slightly different method for sterilizing the surface of puparia was tried by immersing 19 of them in 95 per cent alcohol, then burning this off. These puparia were placed on agar slants and incubated for 48 hours. Six showed contamination and were discarded. The remaining 13 were tested for surface contamination by placing them in broth and later transferring the sterile ones to agar slants. Seven of them showed no growth, either in broth or on agar, and were crushed in broth with a sterile glass rod. Bacterial growth developed from all of them, four yielding *B. carotovorus*.

Another method of isolation consisted in the removal of the contents of the surface-sterilized and tested puparia by a fine-drawn sterilized pipette. Seven puparia picked from rotting cabbage heads in the field were used for this purpose. Five were successfully surface-sterilized. To insure further against contamination a red-hot scalpel was applied to the parts of the puparia in which the sterile pipette was to be inserted. The contents of the puparia were drawn into the pipette and plated on nutrient agar. The bacterial count on all plates was extremely high, and colonies of *B. carotovorus* were numerous on all the plates, corroborating the writer's anticipation that larvae feeding on decaying plants probably ingest large numbers of bacteria, many of them persisting in viable form in the puparia.

On the other hand, there is some evidence to show that maggots developing on the less extensively decayed tissues ingest bacteria in smaller numbers. One hundred maggots which had been gathered from "tunneled" radishes showing little or no decay were allowed to develop on radishes. The puparia thus obtained were surface-sterilized and tested, 41 being found surface-sterile. Eleven of them were crushed in broth and plated, but colonies of *B. carotovorus* were picked from only one. Since it was possible that the pathogene might have been missed in the selection of the colonies, washings were made from the plates and these suspensions inoculated into cabbage-leaf sections. Only one other plate was found to contain the soft-rot organism. The 30 puparia remaining were surface-sterilized, 23 being found surface-sterile. These were crushed in tap water and inoculations made into cabbage leaves; only 2 produced a soft rot. Bacterial counts were low, ranging from 120 to 800. These results suggested that the bacterial content of the insect probably varies according to the bacterial content of the food ingested and that feeding experiments might show definitely the possibility of such a relationship. The results of such experiments are discussed later.

THE IMAGO

Transmission of viable bacteria to the adult fly.—Four adults emerged from the puparia on agar slants while under the observation of the writer. The interior of the empty puparia was swabbed with a tiny cotton swab and inoculations made from this into sterile broth. When this broth was plated on agar bacteria developed on all the plates and the soft-rot pathogene was obtained from two of them. The bacteria apparently had been voided in viable form in the meconium. This was to be expected, as, in the first experiments conducted on the puparia, the exterior of the emerging flies was evidently contaminated with bacteria, for the tracks from the feet and dragging wings of the emerging fly were outlined after a time by bacterial growth. Undoubtedly, the adult fly always is contaminated with bacteria voided during the puparial stage.

The presence of viable bacteria in the meconium suggested that viable organisms might be harbored also in the gastric tract of the adult. To ascertain if this were true 5 male and 13 female flies, collected from rotting cabbage heads in the field, were surface-sterilized by immersion in alcohol, which was then burned off as an additional precaution. The under side of the abdomen was seared with a red-hot scalpel. The digestive tract was dissected out at this point, crushed in broth, and plated on agar. Colonies of *B. carotovorus* were found in 3 of the male flies and 6 of the females. From one of the male flies a culture was isolated which proved identical with *B. areoideae* Towns., and one of the female flies yielded a culture

differing from *B. aeroideae* only in its fermentation of dextrose. Both of these cultures produced rot in cabbage leaves and cabbage plants.

A number of puparia had been reserved for tests for the survival of the pathogene during the winter. Some of these were kept in slightly moistened sand at room temperature, while others were coverd with soil and left outdoors. In February plates were made from the contents of puparia from both lots. Seven out of 12 kept at room temperature contained colonies of *B. carotovorus,* and the same organism was isolated from 11 of the puparia exposed to freezing temperature. Apparently, the soft-rot pathogene is able to survive in the overwintering stage of the insect in Minnesota and can withstand winter temperature here.

Other puparia from the two lots were left undisturbed to await the emergence of the adult. These were used in an effort to determine if the parent of the spring brood can eliminate the soft-rot bacteria in the excrement. While many flies emerged, no excrement was obtained from any of them. However, the gastric tract was dissected out aseptically from many and was frequently found to contain *B. carotovorus.*

Although bacteria of various types, including the soft-rot pathogene, had been found in the meconium and the gastric contents of many of the adult flies, this does not prove the presence of the viable bacteria in the excrement. Accordingly, during the following summer, 74 puparia collected in the field were surface-sterilized, tested, and placed in dry sterile test tubes to allow the adults to emerge. Seventeen flies were obtained, 6 of which deposited excrement on the walls of the test tubes. After the removal of the flies to other sterile tubes, the excrement was moistened with a sterile cotton swab and inoculations made from it into broth from which agar plates were later poured. Bacterial growth developed on all the plates, and colonies of *B. carotovorus* were isolated from 3 of them. The same pathogene was obtained from the gastric contents of 2 of these flies. The 11 remaining flies were aseptically dissected. The gastric tracts so obtained were macerated in sterile tap water from which inoculations were made into cabbage-leaf sections. Soft rot developed from the inoculum of 5 of the flies. These experiments show that the soft-rot pathogene may survive in the gastric tract of the adult fly and that it may be excreted in viable form with the feces. This suggests that the adult also may be a factor in the dissemination of the soft-rot bacteria.

Tests for the presence of the soft-rot bacteria in the eggs.—Leach (11) found that the eggs of the seed-corn maggot could be surface-sterilized without injury to the embryo and that they are free from bacteria internally. It was thought possible that the same condition might obtain in the cabbage maggot. The eggs were first washed carefully to remove all adhering dirt particles. Then they were subjected to a solution of mercuric chloride

(1–1000) for 10 minutes, transferred to sterile broth to rinse off the disinfectant, and finally placed on sterile agar slants planted with surface-sterilized cabbage and radish seed. Three of the eggs floated on the solution and, when examined under the hand lens, were found to be mere shells. The tubes were examined frequently for contamination. Three of them developed a bacterial growth. Eight of the eggs did not hatch, probably having been injured in handling. In 49 of the sterile tubes the maggots crawled over the agar, but no bacterial growth developed in the tracks left by them on the agar, thus indicating that the eggs are free internally from bacterial contamination. The cabbage seedlings which had developed in the tubes during the incubation of the eggs were attacked by some of the larvae but developed no rot. Apparently, the larvae hatched from surface-sterilized eggs are free from bacteria, both internally and externally.

On the other hand, eggs placed without surface sterilization upon sterile agar slants produced bacterial growth on every slant. Bacteria from such growth when used as inoculum often produced soft rot in vegetables. Evidently, the eggs are contaminated externally with both saprophytic and pathogenic types of bacteria. Since it was previously found that the adult fly emerging from the puparium is smeared externally with these various bacteria, it is possible that the eggs may become contaminated in oviposition. It is also possible that such contamination may come from the soil, since, during the season, bacteria corresponding to *B. carotovorus* were isolated from soil samples from Crookston and St. Paul, Minnesota.

THE LARVA

As the newly hatched larvae struggle to free themselves from the egg shells, they may come in contact with the exterior of the shells and bacteria on the shells may thus be transferred readily to the exterior of the larvae. The soil may be a further source of contamination. Larvae hatched from eggs that were not surface-sterilized often produced soft rot in cabbage seedlings. Similarly, larvae hatched from surface-sterilized eggs were transferred to a growth of *B. carotovorus* for a short time, then removed to tubes containing cabbage seedlings; they soon reduced the seedlings to a rotting mass. Maggots were then collected from rotting cabbage heads in the field and placed upon cabbage leaves and seedlings. The large larvae approaching pupating conditions apparently did not feed upon the cabbage but pupated in a day or two and no rot followed. The smaller ones, evidently recently hatched, were very active and fed ravenously upon the food supply, soon bringing about a soft rot. Apparently, the larvae also become contaminated from various sources, the egg shell, the soil, or decaying plant material. The constant boring of the insect into the succulent tissues prevents these from healing over, and the contamination probably is trans-

ferred by the larvae, both from the mouth parts and the body in general to the fresh tissues thus constantly exposed. In this way the maggot serves as an agent of both dissemination and inoculation of the soft-rot pathogene.

PATHOGENICITY OF CULTURES ON CABBAGE PLANTS

Bacterial suspensions in water were made from the cultures obtained from the various stages of the insect. Inoculations were made from these into the stems and leaves of cabbage plants in the greenhouse. All the cultures produced soft rot, although some seemed more virulent than others. In the field it was much more difficult to obtain infection. Repeated inoculations were made, but only 12 per cent of the cultures caused a general collapse of the stems within 2 weeks. The same results were obtained in checks inoculated with *B. carotovorus* and other soft-rot cultures of known virulence. Since the weather was extremely dry, it is possible that a suberization of the tissue occurred before infection could take place. Stump rot developed in many of the inoculated plants later in the season. Among the noninoculated plants stump rot was much less prevalent, indicating that some of the rot appearing late in the season was due to the inoculations but was slow in developing. The same difficulty was experienced in the inoculations with the contents of the puparia and gastric tracts of adult flies, only about 15 per cent of the suspensions that had produced rot on cabbage plants in the greenhouse causing an immediate rot in the field. Again, stump rot was found in most of the inoculated plants later in the season, while very little was found in the noninoculated plants. Figure 1 shows the rot produced in a cabbage plant in the field as a result of inoculation with the contents of a puparium.

PATHOGENICITY ON POTATO STEMS

Inoculations were made with some of the cultures into potato stems and potato sprouts. A soft rot developed in both. While the decaying tissues of the cabbage did not change extremely in color, the decay in some of the potatoes was marked by a dark color varying in intensity according to the variety of potato used and resembling somewhat the discoloration found in potato stems affected with potato blackleg. Since Leach (11) has found that the seed-corn maggot serves as an agent of dissemination and inoculation of the potato blackleg, the idea might suggest itself that the flies from which these cultures were isolated were adult flies of the seed-corn maggot, especially as there is no known morphological difference between the female flies of the two species and both species attack cabbage. However, some of these cultures were isolated from the male fly, and this, as previously mentioned, is readily distinguished from the male fly of the seed-corn maggot.

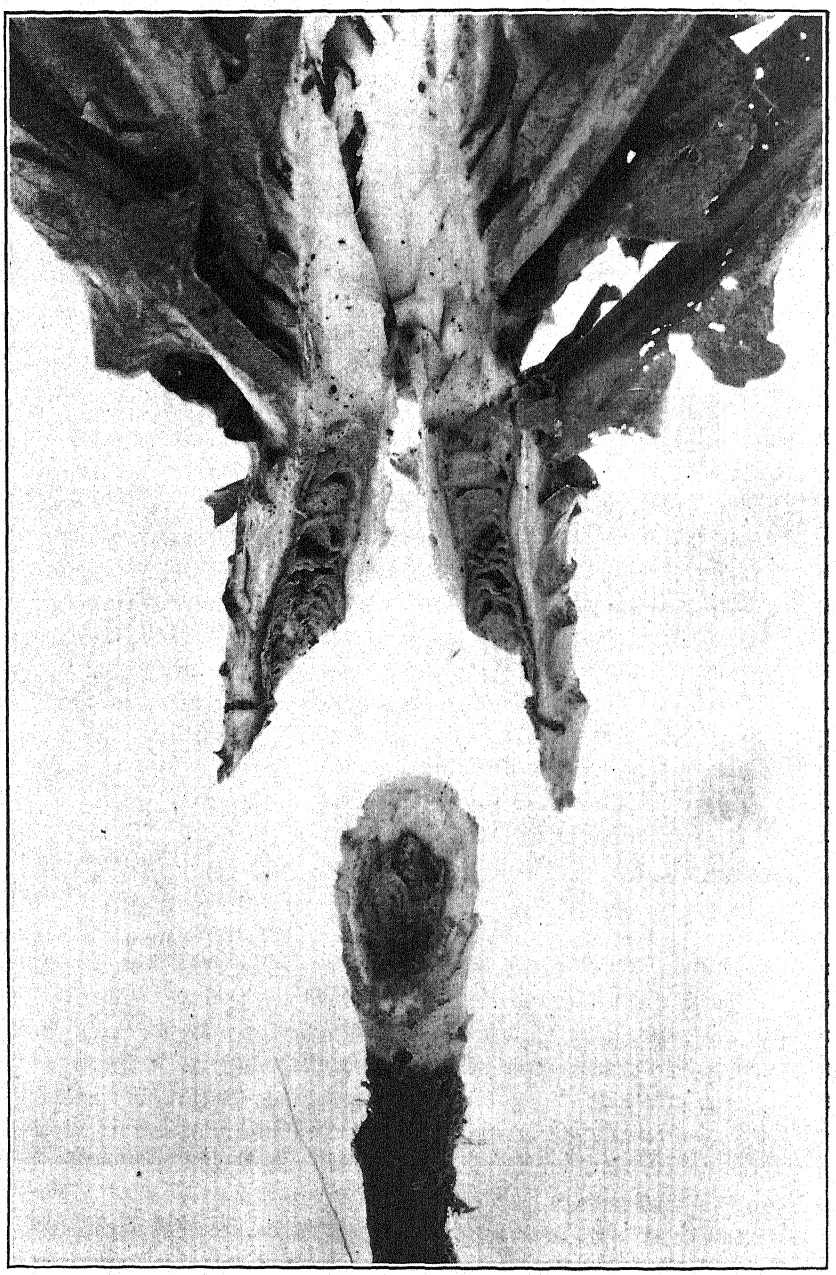


FIG. 1. Stump rot in cabbage developing in the field from inoculation with the contents of a surface-sterilized puparium of a cabbage maggot.

PATHOGENICITY OF B. CAROTOVORUS ON CAULIFLOWER AND OTHER CRUCIFEROUS PLANTS

Different conclusions have been reached by various investigators as to the pathogenicity of *B. carotovorus* on cauliflower and some other Cruciferae. Massey (13) reported, in 1924, that he was unable to produce a soft rot on cauliflower and kohl-rabi with *B. carotovorus.* Jones (10) had originally obtained the same results with his own culture of *B. carotovorus.* However, Harding and Stewart (8) found in 1902 that a culture isolated by them, as well as Jones' culture of *B. carotovorus,* produced a virulent soft rot on both cabbage and cauliflower plants.

The writer inoculated cauliflower heads with tap-water suspensions made from young agar cultures of *B. phytophthorus* Appel, *B. aeroideae* Towns., *B. carotovorus* Jones, and three other cultures of *B. carotovorus* of known virulence. Each of them reduced the cauliflower heads to a rotten mass in a few days. Inoculations were made also into cauliflower, turnip, and kohl-rabi with cultures isolated from the various stages of the cabbage maggot and identified as *B. carotovorus,* and soft rot was produced in all cases. Old cultures in broth were then used for inoculations but with negative results. When these cultures had been rejuvenated by transfer in broth a number of times, soft rot was obtained. Although the old broth cultures used for inoculation were cloudy and seemed to contain a heavy growth of the organism, probably only a few of the bacteria were in a viable condition and were unable to attack the vegetables inoculated before suberization of the tissues took place.

FEEDING EXPERIMENTS WITH LARVAE

According to Riley and Johannsen (14), Celli of Italy, in 1888, fed flies on pure cultures of *B. typhosus* and recovered the organism from the feces and intestinal tract of the insects. Cao (4), another Italian investigator, in 1906, starved cockroaches for 45 days, then fed them with bacteria of the colon-typhoid group and isolated the same type of bacteria from the excrement. Graham-Smith (7) conducted similar experiments on flies in 1910. In 1911 Bacot (1) reared larvae from *Musca domestica,* fed them with cultures of *B. pyocyaneus,* and isolated the organism from the digestive tract of both larvae and imagines. He concluded that pathogenic bacteria may be ingested by the larvae and persist in the adult, with some danger of being distributed in this way.

In the experiments on the cabbage maggot discussed in this paper it seemed apparent that the bacterial flora harbored by the insect varied according to the condition of the food ingested. Therefore, a study of this relationship was undertaken. Eggs were surface-sterilized and placed on sterile slants containing sterile, sprouting cabbage seeds. In a few days a

culture of a fluorescent bacterium was added. The eggs hatched and the larvae grew normally and pupated. The puparia thus obtained were surface-sterilized and the contents withdrawn with a sterile pipette and plated on agar. A high bacterial count of the fluorescent organism was obtained in all of them. Other eggs were placed upon surface-sterilized cabbage leaves, which were then inoculated with *Pseudomonas campestris* (Pam.) E. F. S. This organism also was recovered from the puparia which developed from the larvae. In the same manner, eggs were allowed to hatch on cabbage-leaf sections in petri dishes inoculated with cultures of a fluorescent and a yellow pigmented bacterium. Plates from the puparia obtained showed colonies of both types of bacteria. Similarly, larvae were reared from eggs on cabbage slices inoculated with *B. carotovorus,* and this organism was recovered from the resulting puparia. Bacterial counts made from the puparia in the latter group varied in proportion to the degree of the decomposition of the food consumed. It evidently can be concluded that the bacterial contents of the gastric tract of the larvae depend upon the type of food ingested. The tendency of such bacteria to persist throughout the life cycle of the insect makes it seem probable that the soft-rot pathogene will persist from generation to generation in the cabbage maggot.

OTHER BACTERIA ISOLATED FROM THE INSECT

As previously mentioned, a culture corresponding to *B. aeroideae* was isolated from an adult fly. Another organism, differing from the former only in its fermentation of dextrose, was obtained. Since a single-cell isolation was not made, it is possible that the latter culture may have been contaminated with a dextrose-fermenting organism that could not be removed by repeated platings. Both cultures produced a soft rot on cabbage plants in the greenhouse.

Fluorescent bacteria were always obtained from the insect. These consisted of two types, one a straight, stout, rod-shape organism; the other, slender and curved. Within each of these types variations occurred in the fermentation of various sugars. One of the cultures was identified as *Pseudomonas fluorescens* (Flügge) Migula. Another fluorescent organism, not definitely identified, produced a "water soaked" appearance in cabbage leaves, after which the tissue dried out. This occurred only under high temperature and humidity, so it is probably of no importance economically, since such conditions would not normally exist in storage.

Several times a spore-bearing bacterium, corresponding to *B. mesentericus* Flügge was obtained from puparia. Under conditions of high temperature and high relative humidity it caused a soft rot on cabbage leaves. But, since such conditions usually do not prevail in storage, the organism is probably of no importance in this respect. It did not affect the stems

of cabbage plants in the greenhouse. This bacterium is of some interest, since Brierly (3) has recently reported finding a strain of *B. mesentericus* that is pathogenic for potatoes but does not infect the stems of cabbage seedlings.

Various types of cellulose bacteria were isolated from a number of puparia collected from badly decayed cabbage heads. These included the yellowish pigmented strains of *Spirochaeta cytophaga* (Merker) Hutchinson and Clayton. This was anticipated, for cellulose bacteria were found on the exterior of sound vegetables and were very numerous in decayed cabbage. They probably are ingested in large numbers by the maggot. A yellow pigmented bacterium corresponding to *Ps. campestris* was isolated from the maggot many times. While the cultures showed some pathogenicity for cabbage plants in the greenhouse and may possibly indicate that the cabbage maggot may disseminate and inoculate the organism causing black rot of cabbage, the data obtained are insufficient to justify conclusions at this time.

OTHER INSECTS DISSEMINATING THE SOFT-ROT BACTERIA

Hylemyia antiqua Mg.—Smith and Dickerson (17), in 1907, made the following statement in regard to the onion maggot and its connection with the soft rot of onion: "In feeding, the maggots may attack the onion at the side, but generally they work upward through the center of the bulb, beginning at the bottom, especially when the onion is small. This they can readily do, because the tissue is rather soft and still further softened by the decay caused by the maggot injury." Gibson and Treherne (6) speak of the maggots working "their way down within the sheaths until they reach the young forming bulbs in which they feed and ultimately reduce the same, especially the lower portions, to a rotten mass."

In the course of the research reported here 23 puparia of the onion maggot were successfully surface-sterilized, and crushed in broth. Inoculations were made from 12 of these into onion bulbs and 9 of them produced a soft rot. Plates were made from the remaining 11 puparia and cultures of *B. carotovorus* were obtained from 4. The entire bacterial growth was washed from the 7 plates from which no soft-rot colonies had been isolated; inoculations were made from these suspensions into onion bulbs. Two of them produced the soft rot. Eight puparia were surface-sterilized and placed on sterile agar slants. The tracks left on the agar by the emerging adult flies became outlined by a bacterial growth, and cultures of *B. carotovorus* were obtained from 5 of them.

It is evident from this preliminary investigation that the onion maggot is a factor in the development of the bacterial soft rot of onion. In fact, it is very probable that all of the species of this group of insects will be

found to harbor the soft-rot pathogene and to serve as an agent of dissemination and inoculation of these bacteria.

Cynipids.—Parasites on the cabbage maggot were found to be numerous at the time of this investigation. From 214 puparia, planted on agar slants during the first year of this work, only 104 flies emerged. The rest of the puparia were parasitized by a Cynipid. Although no colonies of the soft-rot organism were found on the plates made from the gastric contents of these parasites, typical soft rot was produced in cabbage plants in the greenhouse from the inoculation of the macerated intestinal tract of many of these insects. The parasite probably had ingested the soft-rot bacteria in feeding upon its host and had retained these in viable form.

Staphylinids.—The puparia of the spring brood of cabbage maggots in 1928 were severely parasitized by a Staphylinid, the parasite emerging from 94 per cent of 494 puparia. The majority of these were *Baryodma bimaculata* Grvh., although several other types, not identified, were found. Four of the parasites emerged from the puparia while observed by the writer. The meconium of these empty puparia was swabbed out and inoculations were made into broth and plated. *Bacillus carotovorus* was isolated from 2 of them. Inoculations from the macerated gastric tract of some of the insects produced soft rot on cabbage plants. The Staphylinid apparently consumes every trace of the contents of the puparium and undoubtedly ingests the soft-rot bacteria present in the pupating maggot, many of these remaining viable in the parasite. Adult Staphylinids emerging from puparia often were found to harbor the soft-rot bacteria. This is probably of no economic importance, since the insect evidently does not attack the plant. Any dissemination of the pathogene it may bring about as a mechanical carrier is undoubtedly limited, while the heavy mortality it causes among the maggots indicates that it is a parasite of considerable importance.

Collembola spp.—Various species of Collembola were frequently found in rotting cabbage. Some of them were transferred to cabbage seedlings growing in sterilized soil, and decay followed their attacks upon the young plants. No attempts were made to ascertain if these insects harbor the soft-rot pathogene in the digestive tract, but they probably serve as mechanical carriers of various bacteria, including those of soft rot. This dissemination may be of no economic importance.

CONCLUSIONS

The cabbage maggot is often associated with the soft rot of cabbage and other vegetables. The egg is free from bacteria internally but is contaminated externally with various types of bacteria from such sources as the soil, decaying plant tissue, and probably the exterior of the parent fly. This contamination often includes the soft-rot bacteria.

The newly hatched larva is also contaminated on the exterior from somewhat similar sources, such as decaying plant material, the soil, and probably the exterior of the eggshell. In feeding upon the cruciferous plants, the maggot inoculates these bacteria, often including the soft-rot pathogene, into the succulent tissues of the host plant, and soft rot may thus develop in varying degrees simultaneously with the attack of the insect upon the plant. In this manner the larva may ingest decaying food tissue containing the soft-rot organisms. Many of these bacteria remain viable in the digestive tract of the maggot.

These organisms also persist in varying numbers inside the puparium and are voided in the meconium in sufficient numbers to contaminate the emerging adult fly. The same type of bacteria survive in the intestinal tract of the fly and are sometimes found in the feces. There is, therefore, a close association between the soft-rot pathogene and the cabbage maggot throughout its entire life cycle. While only a few of the maggots feeding on radishes with no visible decay were found to harbor the soft-rot bacteria, it is probable that the larvae, feeding upon the more soft-rot-susceptible cruciferous plants, ingest enough of these organisms to become an important factor as agents of dissemination and inoculation of the soft-rot bacteria.

In view of these facts it appears that it will be necessary to take into consideration the control of the cabbage maggot in planning measures of control of the soft rot of cruciferous plants.

SUMMARY

1. Entomologists and plant pathologists often have observed a close association between the lacerations of the cabbage plant by the cabbage maggot and the development of bacterial soft rot.

2. A study was made to determine the possibility of the dissemination and inoculation of the soft-rot bacteria by the maggot.

3. The eggs of the insect were found to be free from bacteria internally but contaminated externally with bacteria of various kinds, often including the soft-rot organisms. Such contamination probably is from the body of the parent fly, the soil, or decaying vegetable material.

4. The larvae hatched from surface-sterilized eggs contained no bacteria. However, they apparently become contaminated externally from various sources, such as the soil, decaying plant material, and probably from the contaminated eggshell. They ingest bacteria with their food.

5. The puparia contained viable bacteria of many types, including the soft-rot pathogene.

6. Such bacteria were found also in viable form in the intestinal tract and the excrement of the adult fly.

7. Soft-rot bacteria were found in overwintering puparia and were viable in puparia exposed to freezing temperatures.

8. There is evidently a close association between the soft-rot pathogene and the cabbage maggot in its entire cycle.

9. Cabbage-maggot larvae that had been feeding upon decaying cabbage tissue inoculated fresh cabbage leaves with soft-rot bacteria which reduced them to a rotten mass. The constant lacerations from the maggot prevented the wounded tissue from healing over and checking the decay.

10. Evidently, the larvae of the cabbage maggot, as well as those of closely related species, may serve as agents of dissemination and inoculation of the soft-rot bacteria.

11. Certain insects parasitic on the cabbage maggot were found to disseminate the same type of bacteria, but they are probably of little economic importance in this respect.

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HOST SPECIALIZATION OF BARLEY LEAF RUST, PUCCINIA ANOMALA

E. B. MAINS[1]

INTRODUCTION

Puccinia anomala Rostr. is a rust resembling in many respects the leaf rusts of wheat, *Puccinia triticina* Eriks., and rye, *Puccinia dispersa* Eriks. and Henn. It differs most noticeably from these in that a majority of its teliospores are unicellular, as in the genus Uromyces. Since, however, a small proportion of its teliospores are bicellular it is placed in the genus Puccinia. In other respects it resembles *Puccinia rubigo-vera* DC., having globoid or ellipsoid urediniospores, with scattered germ pores and the telia long covered by the epidermis of the host.

Various other names have been applied to this rust. The name *Puccinia simplex* (Kcke.) Eriks. and Henn. (4) is often used. In the North American Flora, Arthur and Fromme (1) have listed it under the name, *Dicaeoma anomala.*

Compared with the other cereal rusts, this species has received very little study. In Russia, Tranzschel (11) has shown that it produces its aecial stage on *Ornithogalum umbellatum* L. and *O. narbonense* L. He was unable to infect *Muscari botryoides* (L.) Mill., *M. tenuiflorum* Tausch., *Scilla sibirica* Andr. and *Allium angulosum* L. In the United States, Mains and Jackson (8) were able to produce aecia on *O. umbellatum* but not on *Nothoscordium bivalve* (L.) Britton. The aecial stage has been produced also on *O. umbellatum* in Austria (2), Germany (6), and in France (3).

Very little study has been made of the specialization of this rust to grass hosts. For North America, Arthur and Fromme (1) report it only on *Hordeum vulgare* L. The Sydows (10) list it on *H. distichum, H. hexastichum, H. secalinum, H. vulgare,* and *H. zeocrithon.* In Germany, Klebahn (5) observed that in the field *H. distichon, H. distichon nigricans, H. hexastichon, H. zeocrithon, H. coeleste trifurcatum, H. vulgare,* and *H. vulgare cornutum* were heavily rusted, while *H. vulgare nigrum* was free from rust.

Vavilov (12) studied 82 agronomic varieties of barley for susceptibility to *Puccinia anomala.* Five varieties of *Hordeum vulgare hexastichum* L. were more or less susceptible (type 3 or 4). Thirty-nine varieties of *H.*

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vulgare tetrastichum Kcke. were more or less susceptible (type 3 or 4), while 5 were somewhat resistant (type 2½) and one moderately resistant (type 2). Thirty-four varieties of *H. distichum* L. were more or less susceptible, 2 somewhat resistant, 3 moderately resistant, and 1 resistant (type 1).

In Australia, Waterhouse (13) has recently reported studies in which *Hordeum spontaneum* Koch was found to be completely susceptible (type 4). *Hordeum murinum* L. was very resistant (type 0). In a study of 116 varieties of barley, he found 54 varieties of *H. vulgare* L. susceptible (type 4), 10 moderately resistant (type 2), and 15 resistant (types 0–1). In *H. intermedium* Kcke., all eight of the varieties studied were susceptible. In *H. distichon* L., 24 varieties were susceptible and one was resistant. In *H. deficiens* Steud., the four varieties studied were all susceptible.

RESULTS

The studies here reported were begun in 1918. The results obtained in a study of the aecial hosts have already been reported in a previous paper (8). The occurrence of physiologic forms in this rust also has been briefly noted (7). This paper gives in more detail the results obtained in a study of its host specialization.

Wild grass hosts. During the first few years of work on barley leaf rust, attention was devoted largely to a study of the susceptibility of grasses to this rust. In this study 25 to 35 seedlings of several grasses were inoculated in the greenhouse with a number of collections of the leaf rust of barley.

In 1918, a collection of rust obtained by Prof. H. S. Jackson at Ithaca, N. Y., was sown on seedlings of *Agropyron cristatum*[2] Beauv., *A. repens* (L.) Beauv., *A. tenerum* Vasey, *Bromus inermis* Leyss., *B. pratensis* Lam., *B. secalinus* L., *B. sitchensis* Bong., *B. sterilis* L., *B. tectorum* L., *B. villosus* Forsk., *Elymus australis* Scribn. & Ball, *E. canadensis* L., *E. condensatus* Presl., *E. glaucus* Buckl., *Hordeum distichon* L. (Hanna, Hannchen), *H. jubatum* L., *H. pusillum* Nutt., *H. vulgare* L. (Tennessee Winter), *Hystrix hystrix* (L.) Millsp., *Puccinellia nuttalliana* (Schultes) Hitch., *Sitanion hystrix* (Nutt.) J. G. Smith, and *Triticum vulgare* Vill. (Gypsy, Fultzo-Mediterranean). Uredinia were produced in abundance on *Hordeum distichon* and *H. vulgare*. No signs of infection other than occasional faint flecks were noted on the other grasses. This series of grasses was reinoculated twice during the winter with similar results.

Another collection of the leaf rust of barley made by Prof. H. S. Jackson at Madison, Wis., November, 1918, was sown on seedlings of *Agropyron caninum* (L.) Beauv., *A. cristatum*, *A. pseudorepens* Scribn. & Smith, *A.*

[2] Thanks are due Prof. A. S. Hitchcock for identifying and checking the determination of the grasses studied.

repens, A. Smithii Rydb., *A. tenerum, Alopecurus geniculatus* L., *A. pratensis, Bromus altissimus* Pursh., *B. carinatus* Hook. & Arn., *B. erectus* Huds., *B. hordeaceus* L., *B. inermis, B. japonicus* Thunb., *B. Kalmii* A. Gray, *B. polyanthus* Scribn., *B. pratensis, B. secalinus, B. sitchensis, B. tectorum, B. tectorum nudum* Mert. & Koch., *B. villosus, Elymus australis, E. canadensis, E. glaucus, E. virginicus* L., *Festuca elatior* L., *F. rubra* L., *Hordeum distichon* (Chevalier, Hanna), *H. jubatum, H. murinum* L., *H. nodosum* L., *H. pusillum, H. vulgare* (Odessa, Tennessee Winter), *Hystrix hystrix, Puccinella* sp., *Panicularia borealis* Nash., *P. grandis* (S. Wats.) Nash., *Poa pratensis* L., *P. triflora* Gileb; *Sitanion hystrix, Secale cereale* L. (Mammoth Winter), and *Triticum vulgare* (Gypsy, Fultzo-Mediterranean). Uredinia were produced only on varieties of *Hordeum distichon* and *H. vulgare.* No signs of infection were noted other than occasional faint flecking on the other grasses. This series of grasses was reinoculated twice during the winter with similar results.

In 1919, a collection of leaf rust made by the writer at La Fayette, Ind., was sown on seedlings of *Agropyron cristatum, A. tenerum, Elymus canadensis, E. glaucus, Hordeum distichon* (Hanna, Hannchen), *H. pusillum, H. vulgare* (Manchuria, Mariout), *Hystrix hystrix, Sitanion hystrix, Puccinellia* sp., and *Triticum vulgare* (Red Wave). Uredinia developed only on the varieties of *Hordeum distichon* and *H. vulgare.* Slight flecking showed on *Secale cereale, Triticum vulgare,* and *E. canadensis.*

In 1920, leaf rust of barley collected by E. Bethel at San Diego, California, was sown on seedlings of *Hordeum distichon* (Hanna), *H. murinum, H. nodosum, H. pusillum,* and *H. vulgare* (Tennessee Winter). Abundant uredinia were produced on the varieties of *H. distichon* and *H. vulgare,* while only a few faint flecks developed on the other grasses.

A collection of the leaf rust of barley made by the writer at La Fayette, Ind., was sown on seedlings of *Hordeum boreale* Scribn. & J. G. Smith, *H. caespitosum* Scribn., *H. distichon* (Hanna), *H. gussoneanum* Parl, *H. jubatum, H. maritimum* With., *H. murinum, H. nodosum, H. pusillum,* and *H. vulgare* (Oderbrucker, Beardless, Manchuria). Abundant uredinia developed on the varieties of *H. distichon* and *H. vulgare.* The only signs of infection on the other species of Hordeum were faint flecks.

In 1921, a collection of leaf rust of barley made by H. B. Humphrey at Mt. Vernon, Washington, was sown on seedlings of *Hordeum deficiens* (Blackhull), *H. distichon* (Chevalier, Hannchen), *H. gussoneanum, H. intermedium* (Nakano Wase), *H. murinum, H. nodosum, H. pusillum, H. secalinum* Schreb., *H. silvaticum, H. spontaneum* C. Koch, and *H. vulgare* (Manchuria, Tennessee Winter). Abundant uredinia developed on *H. spontaneum* and the varieties of *H. distichon, H. intermedium,* and *H. vulgare.* The other species showed only occasional faint flecks.

These studies would, therefore, indicate that the leaf rust of barley is probably limited to the closely related species *Hordeum deficiens, H. distichon, H. intermedium, H. vulgare,* and *H. spontaneum.*

Physiologic forms of the rust differentiated by barley varieties.—Attention was next directed to a study of the relative susceptibility of barley varieties. Through the kindness of H. V. Harlan and M. N. Pope, of the Office of Cereal Crops and Diseases, an extensive series of over 600 barley varieties was received for study. During the winter of 1923–24, these varieties were given a thorough test in the seedling stage in the greenhouse with a culture of leaf rust received from Washington, D. C. To this collection most of the varieties of *Hordeum distichon, H. intermedium, H. vulgare,* and *H. deficiens* proved to be susceptible. However, a number of varieties especially of *H. vulgare* and *H. distichon* showed resistance. A selected series of these varieties has been studied in succeeding years with other collections of the rust. As a result of these studies, it was found that two physiologic forms of the rust occurred in the United States that could be distinguished by the reactions of certain of these varieties.

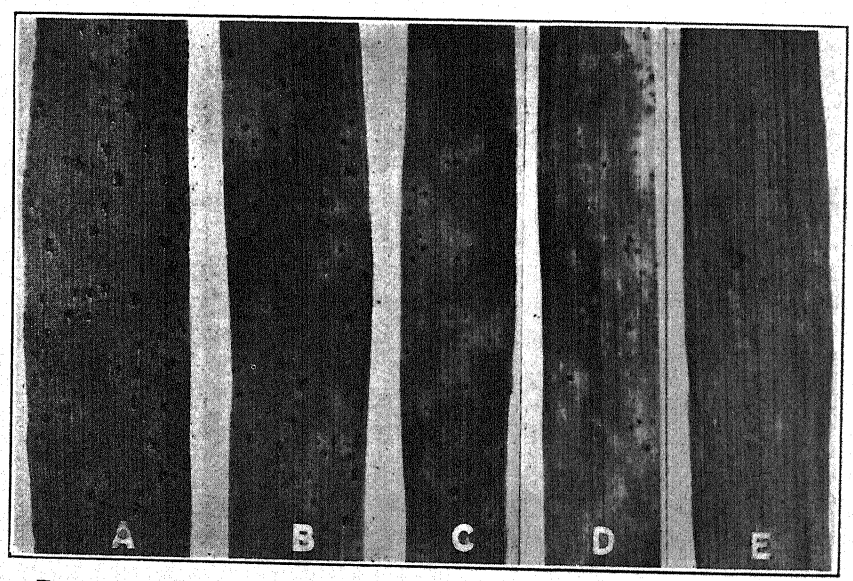


FIG. 1. Types of reaction to leaf rust, physiologic form 1. A, susceptible, type 4, uredinia large, numerous, little or no chlorosis (Wisconsin Winter 49); B, moderately susceptible, type 3, uredinia moderate to large, more or less numerous, usually some chlorosis (Eagle C. I. 913); C, moderately resistant, type 2, uredinia moderate in size, chlorotic and necrotic spots prevalent, frequently without uredinia (Limerick C. I. 1302); D, very resistant, type 1, uredinia small, few, infection mostly evident as necrotic spots without uredinia (Peruvian C. I. 935); E, highly resistant, type 0, no uredinia produced, infection evident only as small necrotic spots or flecks (Horsford C. I. 877).

It is not proposed in this paper to attempt to give all the data accumulated in this study. However, a set of seventeen varieties has been selected which, it is believed, best characterize the two physiologic forms of the rust. Nine varieties which show marked differences in reaction to the two forms, 1 variety susceptible to both physiologic forms, and 7 varieties highly resistant to both physiologic forms have been included.

The methods were similar to those used by Mains and Jackson (9) in their study of the leaf rust of wheat, *Puccinia triticina*. Inoculations were made on seedlings in the second- to third-leaf stage. All studies were made in the greenhouse of Purdue University Agricultural Experiment Station during the winter months.

Five classes of reaction were recognized: Susceptible was denoted by 4 (Fig. 1, A); moderately susceptible, by 3 (Fig. 1, B); moderately resistant, by 2 (Fig. 1, C); very resistant, by 1 (Fig. 1, D); highly resistant, by 0 (Fig. 1, E). These agree with the classes used by Waterhouse (13) in his studies of this rust and also by Mains and Jackson (9) for other leaf rusts of the cereals.

The results of these studies are given in table 1. As is evident from this table, two physiologic forms have been sharply differentiated by the

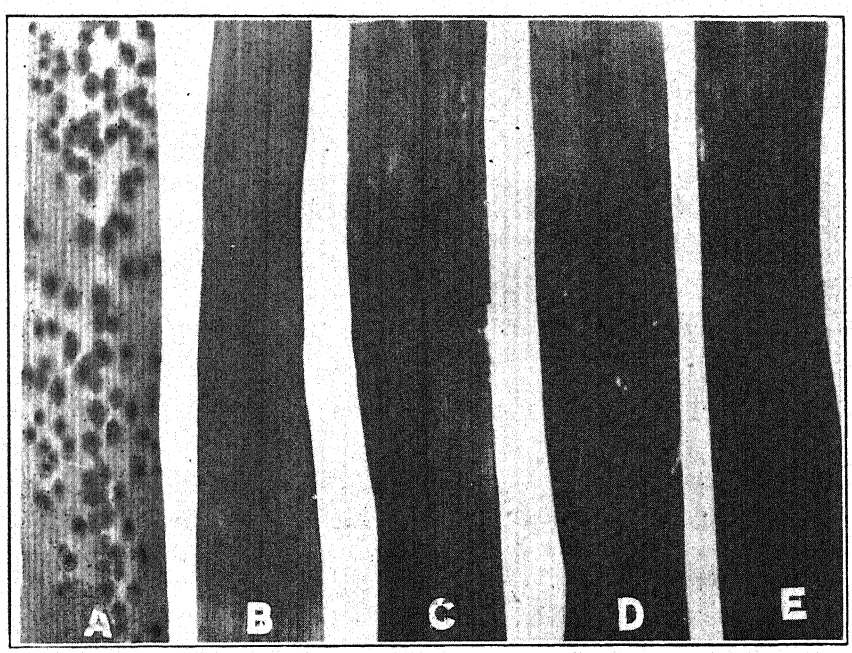


FIG. 2. Reaction of barley varieties to physiologic form 1 of *Puccinia anomala*. A, susceptible, Hanna 906; B, resistant, Beardless C. I. 610; C, Horsford C. I. 507; D, Oderbrucker C. I. 940, and E, Bolivia C. I. 1257. Compare with reaction to physiologic form 2 (Fig. 3).

reaction of Featherston C. I. 1120, Oderbrucker C. I. 940, Oderbrucker C. I. 957, Unnamed C. I. 1347, Malting C. I. 1129, Manchuria C. I. 2330, Hooded Spring C. I. 716, Horsford C. I. 507, and Horsford C. I. 877 (Figs. 2 and 3). These have all been highly resistant to physiologic form 1 and susceptible to physiologic form 2.

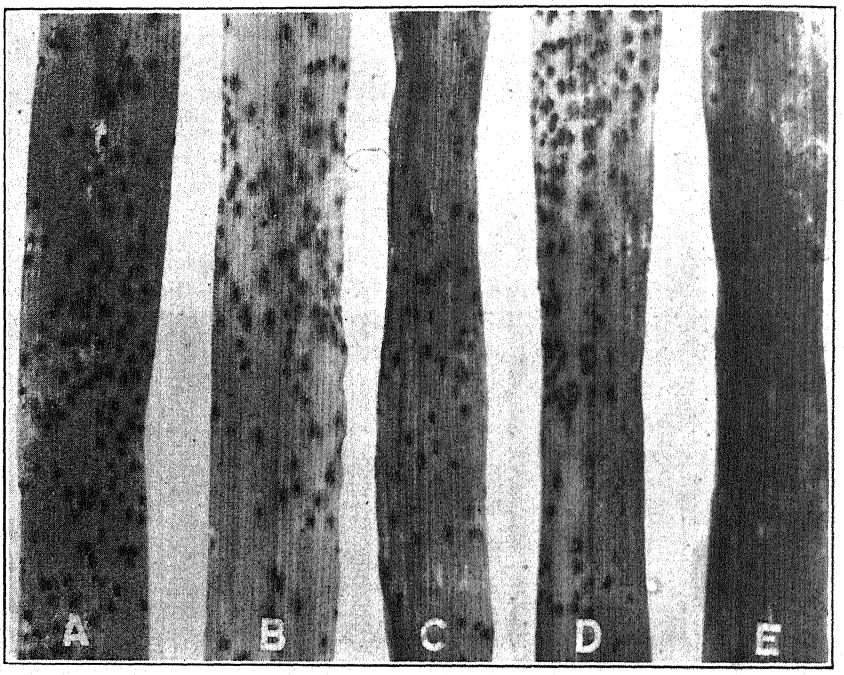


FIG. 3. Reaction of barley varieties to physiologic form 2 of *Puccinia anomala*. A, susceptible, Hanna C. I. 906; B, Beardless C. I. 610; C, Horsford C. I. 507; D, Oderbrucker C. I. 940; E, resistant, Bolivia C. I. 1257. Compare with the reaction to physiologic form 1 (Fig. 2).

Physiologic form 1 was collected as follows:[3] 1923, Arlington, Va.; 1924, Knoxville, Tenn., Arlington, Va.; 1925, Arlington, Va.; 1927, Hubbard, Iowa, Mecklenburg, N. Y., Arlington, Va., La Fayette, Ind.; 1928, San Juan Island, Wash., Atlantic, Iowa, Maumee, Ohio; 1929, Denton, Texas, La Fayette, Ind.

Physiologic form 2 has been obtained from the following localities: 1925, Knoxville, Tenn.; 1927, Knoxville, Tenn., Marshall, N. C., Manhattan, Kans., MacDonald College, Canada; 1928, Arlington, Va., Knoxville, Tenn., Maumee, Ohio, Lewisburg, Ohio.

[3] The writer wishes to express his thanks to H. B. Humphrey, C. E. Leighty, P. W. Rohrbaugh, J. W. Baringer, and C. O. Johnston for their kindness in supplying collections of the rust employed in these studies.

TABLE 1.—*Reaction of seventeen varieties of barley to two physiologic forms of leaf rust, Puccinia anomala*

Variety	C. I. No.[a]	B. P. No.[b]	Physiologic form 1[c]							Physiologic form 2				
			1923	1924	1925	1926	1927	1928	1929	1925	1926	1927	1928	1929
Featherston	1120	320	0	0	0–1	0–1	0–1	0–1	0–1	4	3–4	4	3–4	3–4
Oderbrucker	940	182	1	0	0–1	0–1	0	0–1	0–1	4	3–4	3–4	4	3–4
Oderbrucker	957	193	1	0	0	0–1	0	0–1	0–1	4	3–4	4	3–4	4
	1347	437	0	0	0	0	1	1	0–1	4	4	4	4	4
Malting	1129	326	1	0	0	0–1	0	0	0–1	4	3–4	3–4	3–4	4
Manchuria	2330	538	0	0	0	0	1		0–1	4	4	4–		4
Hooded Spring	716	121	0	1	0–1	0–1	0–1	0	0–1	4–	4	3–4	3–4	4
Horsford	507	48	0	0	0	0–1	0	0	0–1	4	3–4	4	3–4	4
Horsford	877	147	0	0	0–1	0–1	0	0	0–1	4	3–4	3–4	3–4	4
Hanna	906	153	4	4	4	4	4	4	4	4	4	4	4	4
Callas	2440	604	2	1	1	0–1	0–1	0–1	0–2	1	1	1	0–1	1–2
Mecknos Moroc	1379	463	1	0	0	0	1	0–2	0–1	0	0–1	1	0–1	1–2
Peruvian	935	178	1	1	0–1	0–1	0–2	0	0–1	0–1	1–2	1–2	0	0–2
Quinn	1024	247	2	0	0	0	0–1	0	0–2	0	0	0	0	0–1
Bolivia	1257	375	1	0	0	0	0	0	0	0	0–1	0	0	0–1
Juliaca	1114	314	0	0	0–1	0	0–1	0–2	0–1	0	0–1	0	0	0–1
	2329	537	2	1	1	0–1	0–1	0–1	0–1	0	0	0	0–1	0–1

[a] Accession number of the Office of Cereal Crops and Diseases of the U. S. Department of Agriculture.

[b] Accession number of the Department of Botany, Purdue University Agricultural Experiment Station.

[c] Rust collected during the summer of the years listed below and studied during the following winter months. The results for all collections of each year are summarized in each column.

A mixture of the two physiologic forms occurred in only one collection, that from Maumee, Ohio, in 1928. Collections from La Fayette, Ind., have not been included for the most part, since cultures of the two forms have been introduced into the experimental plots in studies of varietal susceptibility. Consequently, the forms present did not indicate natural distribution.

It is interesting to note the occurrence of physiologic forms at Arlington, Va., and Knoxville, Tenn. Physiologic form 1 was prevalent at Arlington, Va., in 1923, 1924, 1925, and 1927, while physiologic form 2 occurred in 1928. At Knoxville, Tenn., physiologic form 1 occurred in 1924, while physiologic form 2 was prevalent in 1925, 1927, and 1928.

Waterhouse (13), in his study of barley varieties for reaction to *Puccinia anomala*, in Australia, found that 16 out of 116 varieties showed considerable resistance and 10 were moderately resistant in studies of the seedlings during the winter months. The same author (14) has recently shown that during the summer months a number of these are susceptible.

TABLE 2.—*Reaction, to physiologic forms 1 and 2 of leaf rust in the United States, of barley varieties resistant in Australia*

Variety	Australian No.	B. P. No.	Physiologic form 1		Physiologic form 2		Australian[a] rust
			1928	1929	1928	1929	
California Feed	B59	799	4–	2–3	4	3	[illegible]
Psakwon	B81	800	4	4	4	4	[illegible]
Locride	B86	801	4	3	3–4	4	[illegible]
Sahara	B95	802	4	3	4	2	[illegible]
Coast	B121	803	4	4–	4–	3	[illegible]
Marionet	B113	804	4–	4–	2–3	3	[illegible]
Orge Fourrager	B102	805	1	1	2	1	[illegible]
Lion	B33	806	4	4	4	4	[illegible]
Smooth Awn × Luth	B34	807	4	4	4–	4	[illegible]
Smooth Awn × Manchuria	B35	808	4–	4	2	4	[illegible]
C. I. 2208		809	2	0	3	4	0–1
Virginia Hooded	B6	810	2+	1	3	2–3	0–1
Smooth Awn × Manchuria	B36	811	2	2	3+	3–4	0–1
Smooth Awn × Manchuria	B37	812	2+	2	3+	4	0–1
Smooth Awn × Manchuria	B38	813	2	1	3+	4	0–1
Manchuria Sel. C163	B40	814	2+	1	4	4	0–1
Manchuria Sel. C168	B41	815	2+	2	3+	4	0–1
Manchuria Minn. 184	B43	816	2+	1	3+	4	0–1
Manchuria	B44	817	2+	1	4	3–4	0–1
O. A. C. 21	B61	818	2+	2	4–	4	0–1
No. 22	B69	819	1–2	0	2	2	0–1
No. 305	B85	820	3	2–3	3	2	0–1
Colsess	B97	821	2	0	3–4	4	0–1
Orge 4th	B100	822	0	0	0	0	0–1
Orge 14 J	B101	823	0	0	0	0	0–1
C. I. 2220	B23	824	2+		4		0

[a] Reaction given by W. L. Waterhouse. Jour. Proc. Royal Soc. N. S. Wales **61**: 223–225. 1928.

Mr. Waterhouse kindly furnished the writer with seed of these 26 varieties and these have been studied for reaction to physiologic forms 1 and 2 during the winters of 1928–29 and 1929–30. The results of these studies are given in table 2 in comparison with those obtained by Waterhouse with the Australian rust under similar conditions.

The results would indicate that the rust of Australia is a third physiologic form. Most of the varieties were more susceptible to physiologic forms 1 and 2 than reported by Waterhouse. The Australian rust approached physiologic form 1 in that all the varieties highly resistant to it also showed some resistance to physiologic form 1, although to a less extent.

Four varieties, Orge Fourroger B102, No. 22 B69, Orge 4th B100, Orge 14J B101, were resistant to all of the forms studied in Australia and the United States. The last two were very resistant to both physiologic form 1 and 2 as well as the Australian rust.

SUMMARY

1. In these studies favorable hosts for the leaf rust of barley, *Puccinia anomala*, were found in only a few closely related species of Hordeum, viz.: *H. vulgare, H. deficiens, H. distichon, H. intermedium,* and *H. spontaneum.*

2. Seedlings of *Agropyron caninum, A. cristatum, A. pseudo-repens, A. repens, A. Smithii, A. tenerum, Alopecurus geniculatus, A. pratensis, Bromus altissimus, B. carinatus, B. erectus, B. hordeaceus, B. inermis, B. japonicus, B. pratensis, B. secalinus, B. sitchensis, B. sterilis, B. tectorum, B. tectorum nudum, B. villosus, Elymus australis, E. canadensis, E. condensatus, E. glaucus, E. virginicus, Festuca elatior, F. rubra, Hordeum boreale, H. caespitosum, H. gussoneanum, H. jubatum, H. maritimum, H. murinum, H. nodosum, H. pusillum, H. secalinum, H. silvaticum, Hystrix hystrix, Panicularia borealis, P. pratensis, P. grandis, Poa pratensis, P. triflora, Puccinellia nuttalliana, Secale cereale, Sitanion hystrix,* and *Triticum vulgare* showed little or no signs of infection beyond occasional slight flecking.

3. Two physiologic forms of *Puccinia anomala* have been distinguished in the United States. These are distinguished by the differences in reaction of a select set of barley varieties, Featherston C. I. 1120, Oderbrucker C. I. 940, Oderbrucker C. I. 957, Unnamed C. I. 1347, Malting C. I. 1129, Manchuria C. I. 2330, Hooded Spring C. I. 716, Horsford C. I. 507, and Horsford C. I. 877, which are highly resistant to physiologic form 1 and more or less susceptible to physiologic form 2.

4. Seven varieties, Callas C. I. 2440, Mecknos Moroc C. I. 1379, Peruvian C. I. 935, Quinn C. I. 1024, Bolivia C. I. 1257, Juliaca C. I. 1114, and unnamed C. I. 2329, were found to be more or less resistant to both physiologic forms.

5. Of a series of 26 varieties, found by Waterhouse to be resistant to *Puccinia anomala* in Australia, 22 varieties were more susceptible to the physiologic forms from the United States, indicating the existence of a third physiologic form in Australia.

6. Four varieties from Australia, Orge Fourrager B102, No. 22 B69, Orge 4th B100, and Orge 14 JB101, were resistant to the physiologic forms used in these studies as well as to the rust in Australia.

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STUDIES ON THE PROPERTIES OF THE BEAN-MOSAIC VIRUS

T. G. FAJARDO[1]

The determination of the properties of different plant viruses now occupies an increasingly important position with respect to their classification. The properties of the ordinary tobacco-mosaic virus and that of cucumber mosaic, for example, are now well known (7, 4), but, up to the present, nothing has been reported concerning certain properties of the bean-mosaic virus. This may be because bean mosaic is difficult to transmit artificially, a difficulty frequently encountered by the earlier workers. A method of artificial inoculation has been developed by the writer (5), however, that has given a fairly high percentage of infection and has made possible a preliminary study of the properties of this virus. The investigation has been conducted in relation to the following phases: (1) Effect of aging on the virus *in vitro*, (2) effect of dilution, (3) resistance to alcohol treatments, (4) resistance to drying in plant tissues, (5) thermal death point, (6) effect of dry heat on the virus in infected seeds, (7) effect of freezing, and (8) filterability through bacterium-proof filters.

METHODS

The methods followed in these studies were uniform unless otherwise stated. The bean-mosaic virus was obtained in plant extract either by grinding up mosaic bean plants in a sterilized mortar with sterile sand or by passing diseased material through a fine meat chopper. The juice was then separated from the macerated tissues by squeezing it through cheesecloth. After the various treatments, the juice was artificially inoculated to young healthy seedlings of the variety Rogers Stringless Green Pod or Refugee 1000–1, both of which are late susceptible varieties. The inoculation was performed by rubbing or mutilating the two simple leaves of the plants with a small piece of cheesecloth dipped into the juice, to which a small quantity of sterilized sand had been added. The control plants were inoculated by the same method with a portion of the same but nontreated plant extract. The plants were kept in a greenhouse at a temperature of 24–28° C. for 25 days. Under ordinary greenhouse conditions, infected plants develop typical mosaic symptoms 15 to 20 days after inoculation.

RESULTS

(1) *Effect of aging on the virus in vitro.* The results of a series of experiments have shown that the virus of bean mosaic loses its power of

[1] The writer is indebeted to Professor L. R. Jones, under whose encouragement the work was pursued, and to Drs. J. Johnson and I. A. Hoggan, who kindly read and criticized the manuscript during its preparation.

infection rapidly in plant extract. Undiluted juice, whether allowed to stand in bulk or in 5 c.c. portions at room temperatures, failed to cause infection after 24 hours. Juice diluted with sterile water under the same greenhouse conditions loses its infectiousness more rapidly. Dilutions of 1 part of plant extract to 10 parts of water remained infectious after 14 hours, while dilutions of 1 to 50 and 1 to 100 failed to produce infection after this time. The percentage of infection decreased markedly with increase in dilution and duration of aging.

(2) *Effect of dilution.* The results of four experiments indicate that the bean-mosaic virus loses its infectious properties readily on dilution with distilled water, without aging. A low percentage of infection was obtained when the virus was diluted in plant extract to 1 to 1000. No infection was secured at dilutions higher than 1 to 1000.

(3) *Resistance to alcohol treatments.* The virus of bean mosaic is very sensitive to alcohol. A low percentage of infection was obtained after treatment of the virus with 50 per cent alcohol at room temperatures for less than five minutes, while no infection resulted after treatment with 75 per cent alcohol or higher strengths for the same length of time. No infection was obtained after treatment with 25 per cent alcohol for 30 minutes at room temperature.

(4) *Resistance to drying in plant tissues.* The bean-mosaic virus is also very sensitive to drying and loses its infectious properties rapidly on drying. Cut young mosaic plants were allowed to wilt or dry at room temperature for various lengths of time, each sample being weighed at the beginning and end of the period. The loss in weight was then replaced by addition of distilled water, the plants macerated, and the extract obtained inoculated immediately to young plants. Table 1 shows the results of two such experiments and indicates that the virus was still infectious in plants that had been allowed to dry for 48 hours. No infection was secured from plants after drying 72 hours. Young mosaic plants kept turgid in an ice box, however, were still infectious after 72 hours. The virus, therefore, lives longer in plant tissues than in plant extract but becomes inactivated when the tissues dry out.

(5) *Thermal death point.* The bean-mosaic virus has a relatively low thermal death point. Five and 10 c.c. portions of mosaic plant extract were heated for 10 minutes in thin-wall test tubes in a water bath. After heating at 44–46° C., a single infection resulted in 10 plants inoculated, while no infection obtained after heating at 55–56° C. The thermal death point for the virus of bean mosaic therefore appears to lie between 44° C. and 56° C., probably close to 46° C.

(6) *Effect of dry heat on the virus in infected seeds.* Several tests with mosaic seeds of the varieties Rogers Stringless Green Pod and Refugee

TABLE 1.—*Resistance of the bean-mosaic virus to drying at room temperature*

Inoculum	Duration of aging	Loss of weight	Plants infected of 5 inoculated
	Hours	*Per cent*	*No.*
Series I			
1 (Control)	0	0.0	5
2	4	35.7	5
3	12	53.6	4
4	24	66.7	1
5	48	80.0	1
6	72	82.6	0
7	96	86.4	0
Series II			
1 (Control)	0	0.0	4
2	16	50.0	4
3	24	65.0	3
4	48	84.0	1
5	72	87.5	0
6	96	90.0	0

1000–1, heated 12 and 24 hours, respectively, at various temperatures in thermo-regulated ovens, showed that the virus could withstand higher temperatures in dry seeds than in plant extract. A certain number of mosaic seedlings were obtained from seeds heated at 65–66° C. for 24 hours, while seeds heated at 80° to 85° C. failed to germinate. Water extracts of the seeds heated at 80°–85° C. gave no infection when inoculated to young bean plants. These results, summarized in table 2, show that the highest temperatures which allow of subsequent germination do not destroy the virus in the seed. This is in agreement with the conclusions reached by Reddick and Stewart (10).

(7) *Effect of freezing.* Freezing undiluted, freshly expressed juice of mosaic plants at temperatures of –7° to –10° C. did not appear to affect the viability of the virus. The percentage of infection was found to decrease with the duration of freezing, until after 24 hours no infection resulted. The results, however, were similar to those obtained when testing at room temperature the effect of aging on the virus. It is, therefore, believed that the freezing, itself, did not cause inactivation of the virus.

(8) *Filterability through bacterium-proof filters.* The bean-mosaic virus, as far as could be determined, failed to pass through any grade of Berkefeld filter at a reduced pressure. In these experiments, inoculation of the first 5 to 10 c.c. of the filtrate passing through a grade V, coarse Berkefeld filter gave no infection. The nonfiltered juice was found to be still highly infectious and gave 100 per cent infection.

TABLE 2.—*The effect of dry heat on the bean-mosaic virus in infected seeds*

Variety	Time and temperature of heating	Seeds planted	Germination	Mosaic infection
		No.	*Per cent*	*Per cent*
	13 Hours			
	24° C.	20	95	58
Rogers	44–46° C.	20	60	67
Stringless	65–68° C.	40	80	59
Green Pod	80–85° C.	20	0	0
	85–90° C.	20	0	0
	24 Hours			
	24° C.	20	75	73
	44–46° C.	20	85	71
	65–66° C.	40	90	72
	80–85° C.	20	0	0
	85–90° C.	20	0	0
	13 Hours			
	24° C.	20	90	94
Refugee	44–46° C.	20	85	88
1000–1	65–66° C.	40	58	82
	80–85° C.	20	0	0
	85–90° C.	20	0	0
	24 Hours			
	24° C.	20	95	84
	44–46° C.	20	90	83
	65–66° C.	40	25	60
	80–85° C.	20	0	0
	85–90° C.	20	0	0

A COMPARISON OF THE PROPERTIES OF THE BEAN-MOSAIC VIRUS WITH THOSE OF CERTAIN OTHER VIRUSES

These results indicate that bean mosaic is caused by a specific virus, distinct from other viruses that have been similarly studied, such as the virus of tobacco mosaic, of cucumber mosaic, and of potato-crinkle mosaic. This is readily seen from table 3. While the tobacco-mosaic virus may remain infectious in plant extract for 1 or more years (3), the bean-mosaic virus apparently retains its infectiousness only about 20–24 hours, in this respect resembling more closely the viruses of cucumber mosaic and potato-crinkle mosaic, which lose their power of infection in 24–48 hours and in 24 hours, respectively (4, 8). The virus of bean mosaic retains its infectious nature in dried mosaic tissues between 2 and 3 days; that of tobacco mosaic for more than 1½ years (1); and that of cucumber mosaic (4), less than 10 days.

Again, while the bean-mosaic virus gives no infection at dilutions higher than 1–1000, the tobacco- and cucumber-mosaic viruses are still infectious at dilutions of 1 to 10,000 (2, 4). On the other hand, the crinkle-mosaic virus produces little infection at dilutions above 1 to 10 (8) and is, in this respect, more sensitive than the bean-mosaic virus.

The bean-mosaic virus is relatively sensitive also to alcohol. No infection was obtained after treatment with 25 per cent alcohol for 30 minutes, while the tobacco-mosaic virus resists 50 per cent alcohol for several days and 80 per cent alcohol for half an hour or less (3). Johnson (7) obtained no infection with the cucumber-mosaic virus after treatment with 50 per cent alcohol for 1 hour.

The viruses also differ in their thermal death points, that of bean mosaic lying between 44° and 56° C., probably close to 46° C., that of tobacco mosaic being about 90° C. (9), that of cucumber mosaic 75° C. (4), and that of potato-crinkle mosaic 43° C. (8).

No success was obtained in attempts to filter the bean-mosaic virus through coarse Berkefeld filters, the virus being in this respect comparable to that of crinkle mosaic. The tobacco-mosaic virus has been shown by Iwanowski (6) and others to pass readily through bacterium-proof filters, and the cucumber-mosaic virus has been shown by Doolittle (4) to pass through a medium Berkefeld filter.

TABLE 3.—*Comparison of the properties of the bean-mosaic virus with those of the viruses of potato, tobacco, and cucumber mosaics*

Property of virus	Source of virus			
	Bean mosaic	Potato "crinkle mosaic"[a]	Tobacco mosaic[b]	Cucumber mosaic[c]
Resistance to aging *in vitro*	20–24 hrs.	24 hrs.	1 or more yrs.	24–48 hrs.
Tolerance to dilution	1–1000	1–10	1–10,000	1–10,000
Resistance to alcohol	25% for 30 min.	——	80% for 30 min.	50% for 1 hr.[a]
Resistance to aging in dried plant tissues	48–72 hrs.	——	1 or more yrs.	Less than 10 days
Thermal death point	Probably close to 46° C.	43° C.	90° C.[d]	75° C.
Filterability through coarse Berkefeld filter	Not filterable	Not filterable	Filterable	Filterable

[a] Determination by Johnson.
[b] Determination by Allard except as noted.
[c] Determination by Doolittle except as noted.
[d] Determination by Mulvania.

SUMMARY

(1) The virus of bean mosaic differs in its properties from the viruses of tobacco mosaic, cucumber mosaic, and potato-crinkle mosaic.

(2) The bean-mosaic virus retains its infectiousness for 20–24 hours in plant extract, when aged at room temperatures. In diluted juice, the virus loses its infectiousness sooner than in nondiluted juice.

(3) At a dilution of 1–1000 with distilled water, mosaic-plant extract gives a low percentage of infection, but at higher dilutions no infection has been obtained.

(4) The virus is inactivated by treatment with 25 per cent alcohol for 30 minutes and with 75 per cent alcohol for less than 5 minutes.

(5) The virus is very sensitive to drying in plant tissues, since infection was obtained from mosaic seedlings that had been allowed to dry at room temperatures for 72 hours.

(6) The thermal death point of the virus appears to lie between 44° and 56° C., probably close to 46° C.

(7) The virus is able to resist higher temperatures when in the seed than in expressed juice. It was not destroyed at temperatures that did not destroy the germinating capacity of the seed.

(8) Freezing apparently does not affect the viability of the virus.

(9) The virus was not found to pass through any grade of Berkefeld filter.

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REPORT OF THE COTTON-ROOT-ROT CONFERENCE AT TEMPLE, TEXAS

WALTER N. EZEKIEL AND D. C. NEAL

The third annual conference of workers engaged in the study of cotton root rot, caused by *Phymatotrichum omnivorum* (Shear) Duggar, was called by Director A. B. Conner of the Texas Agricultural Experiment Station at the Kyle Hotel, Temple, Texas, on January 15, 1930. Director Conner presided at the morning session and Dr. A. G. McCall, Chief of Soil Investigations, Bureau of Chemistry and Soils, U. S. Department of Agriculture, at the afternoon session. The meeting was attended by about 45 people, 12 of whom were from the U. S. Department of Agriculture, 15 from the Texas Agricultural Experiment Station, and the remainder press representatives and other nontechnical persons interested in the problem. Approximately 40 papers were presented; these are summarized below in the order of presentation.

Occurrence, losses.—J. J. Taubenhaus, Texas Agricultural Experiment Station, reported that root rot has now been found in 169 counties of Texas and that it has been found for the first time in Arkansas. The estimated annual aggregate loss caused by the disease in Texas, considering all crops, is approximately $100,000,000. Of this, over $60,000,000 is estimated as due to the 15 per cent average reduction in yield of cotton.

New hosts.—S. E. Wolff, Texas Agricultural Experiment Station, reported that *Allionia lanceolata* and *Psoralea tenuiflora* were found to be highly susceptible. One plant of *Salvia greggii* succumbed to root rot, the first instance reported of a mint dying from this disease. Taubenhaus summarized tests at College Station in which pomegranate, live oak, and one species of hackberry were found resistant; while yaupon and another species of hackberry were susceptible. W. J. Bach, Texas Agricultural Experiment Station, reported the following new hosts from Weslaco: anise, endive, escarole, jacaranda tree (*Jacaranda mimosaefolia*), *Schinus terebinthifolius,* and *Zelkova sinica.* The Turks-cap hibiscus (*Malvaviscus conzattii* (*arboreus*)) appeared resistant in spite of repeated inoculation. This ornamental plant is the only member of the Malvaceae known to be resistant to root rot.

Soil relations.—W. T. Carter, Soil Survey, discussed the distribution of root rot in Texas as related to soil types. Paul R. Dawson, Bureau of Chemistry and Soils, reported further on field observations in which small root-rot areas, occurring in predominantly noninfested fields of Wilson

and Irving clay soil, were closely associated with localized spots or streaks of shallow soil. These streaks were alkaline, pH 7.4 to 8.3, contained numerous concretions of calcium carbonate, and were very low in organic matter; while the noninfested areas were predominantly acid, pH 5.8 to 6.25, free of calcium carbonate, and appreciably higher in organic matter.

MORPHOLOGY AND PHYSIOLOGY OF THE FUNGUS

Taubenhaus reported studies of spore mats in which direct morphologic connections were found between the hyphae bearing the Phymatotrichum conidia aboveground and the typical Ozonium strands on roots below ground.

D. C. Neal, Bureau of Plant Industry, found that in soil cultures containing dextrose, lactose, and corn meal, considerable growth obtained but no sclerotia formed. Such competitive organisms as Aspergillus, Penicillium and *Fusarium* spp. partially arrested the development of strands of *P. omnivorum* in agar plates inoculated simultaneously. W. N. Ezekiel, Texas Agricultural Experiment Station, reported artificial culture of *Phymatotrichum omnivorum* on purely synthetic media and the production of sclerotia in such cultures.

Temperature, moisture relations.—Neal summarized experiments conducted in cooperation with W. W. Gilbert on the temperature relations of *P. omnivorum.* The minimum, optimum, and maximum temperatures for sclerotia formation appear to be 18°, 29°, and 36° C., respectively. B. F. Dana, Texas Agricultural Experiment Station, reported that soil temperatures at Temple, Texas, favor root rot from May to October, and that during this period the prevalence of the disease varies with soil-moisture conditions. Neal found that sclerotia developed in cultures in Wilson clay soil maintained at 20 per cent, 30 per cent, and 40 per cent soil moisture on an oven-dry-weight basis and that the optimum was between 35 per cent and 40 per cent.

Ezekiel reported an experiment planned to explain absence of root rot on bottom lands. Roots cut from freshly wilted cotton plants were stored in large crocks from which some were removed periodically to test the virulence of the Phymatotrichum strands on the roots by inoculation of normal cotton plants. In flooded soil, at room temperature, the fungus was virulent after three days but not after a week; in moist air, at room temperature, for two but not three weeks; and in moist air in the refrigerator, for three but not four weeks. C. J. King, Bureau of Plant Industry, found that 20 per cent of individual sclerotia of *P. omnivorum* were still viable after being immersed in water for 121 days.

LIFE HISTORY

Summer spread.—H. C. McNamara and D. R. Hooton, Bureau of Plant Industry, reported that root rot had spread in the fields of the U. S. Cotton Breeding Station at Greenville, Texas, an average of about 10 feet a year. Maps of the distribution of root rot since 1920 showed that the spots passed through cycles of increase culminating in almost complete kill, followed by abrupt decreases, often in a single year, to a few scattered areas of infection.

Winter spread, overwintering.—Taubenhaus presented studies to show that root rot spreads, not only in summer, but also on the roots of plants during the fall and winter months. Numerous experiments indicate that the fungus overwinters on infected, live roots of cotton and other susceptible plants rather than on decayed roots.

Sclerotia.—King, who first observed the production of sclerotia in cultures in the laboratory, and Neal reported separate results in which sclerotia were still viable in cultures a year old. The production of sclerotia in the laboratory in soil-filled containers was discussed by several workers. Sclerotia were first found in the field by Neal in May, 1929, and were later found by Taubenhaus and Dana. Many primary infections during 1929 were traced to the sclerotia, particularly in fields that had been in clean fallow and in grain rotation during previous years. Neal reported that 132 sclerotia, of which 90 per cent were still viable, were collected at a depth of 18 inches beneath a single cotton plant, a primary infection center in a field at San Antonio. Cultures were readily obtained from sclerotia collected in the field. Successful inoculations of cotton plants with sclerotia were reported by King, Neal, and Taubenhaus. King and Neal obtained good infection with cultures of *P. omnivorum* which had been grown on dead plant tissues and other nutrient media 11 and 12 months, respectively, prior to the inoculation; and Neal and Taubenhaus obtained infection with cultures isolated originally from sclerotia collected in the field. Taubenhaus found that the same sclerotia could grow as often as five times after the previous growth had been removed. King reported the development of a second crop of sclerotia in 4 to 5 months by budding from old clusters in cultures of sand and cotton roots that had been buried in the soil during the winter months and in similar cultures stored in the laboratory at room temperature. Small, individual sclerotia, air-dried at room temperature, were no longer viable after about $1\frac{1}{4}$ hours. Neal found similarly that single sclerotia, dried on filter paper for 6, 12, and 24 hours, respectively, at room temperature, were no longer viable. Conglomerate masses of sclerotia retained their viability after air drying for 20 hours in a calcium chloride desiccator but were killed after three days. Large hyphae in conidial mats, collected originally in an alfalfa field, retained their viability after air drying in the laboratory $6\frac{1}{2}$ months.

King described the growth of *P. omnivorum* in a long glass tube containing sterile moist sand and small deposits of sterile dead cotton plant tissues placed at intervals of 2½ feet. The strands grew 11 feet in 149 days. Sclerotia developed in the strands soon after the terminal growth had advanced a few centimeters beyond the first, second, and third deposits of root tissues, but none were produced during the last 3 months of growth. Live root-rot strands were commonly found on dead tree roots several years after the trees had been removed; the roots of date palms and other plants penetrating these softened tissues were infected.

CONTROL EXPERIMENTS

Soil reaction.—Continued studies at the Texas Agricultural Experiment Station on the effect of soil reaction on root rot were summarized by Ezekiel. In a number of inoculation experiments, there were higher percentages of infection, higher percentages of plants killed, and a shorter average interval between inoculation and wilting in alkaline or neutral soils than in acid soil. Root rot was not controlled by acidification of the surface layer of soil, only. G. S. Fraps, Texas Agricultural Experiment Station, mentioned the development of a method by which the amount of acid necessary to change the soil to a desired degree of acidity may be determined. H. E. Rea, of the same station, reported field tests with sulphur in which root rot was partially controlled by high rates of application; however, there was considerable acid injury to cotton.

Rotation and clean-culture experiments were summarized by H. Dunlavy and Rea, of Texas, and McNamara and Hooton, and G. T. Ratliffe and I. M. Atkins, Bureau of Plant Industry. The results of these experiments were somewhat erratic. Clean tillage or rotation with nonsusceptible crops for one year resulted in occasional control, while two and three years of similar treatment have in some cases appreciably reduced infection.

Subsoiling experiments conducted independently by a number of investigators (Dawson, Dunlavy, McNamara, Neal, and Ratliffe and Atkins) yielded promising results in 1929. In most cases there was less root rot on subsoiled land than in adjoining check plots.

Fertilizer experiments in the black-land region of central Texas were reported by Dawson and H. V. Jordan, Bureau of Chemistry and Soils. In general, significant increases in yield resulted from the application of mixtures containing the higher proportions of phosphate together with some nitrogen, although in some instances the greatest response was to the higher proportions of nitrogen in the presence of phosphate. The increases in yield were in some cases in excess of 75 per cent and counterbalanced losses due to root rot. In several experiments, phosphatic fertilizers hast-

ened maturity; the yields were increased as bolls matured before the plants were killed by root rot.

Soil disinfectants.—King found that 1 per cent Formalin solution and 2 per cent cresylic acid were effective in checking the spread of the fungus in small isolated spots in alfalfa fields, but, in a few cases, infection reappeared in later years. The incidence and extent of infection were reduced in infested areas, flooded with these solutions before cotton was planted. Several small spots of root rot appeared late in the season and indicated failure of the treatment to destroy all sources of infection. Dana reported unsuccessful results from repeated treatments with formaldehyde in field plots. Applications of Semesan around shrubs in root-rot areas gave partial control. Bach obtained good results with copper sulphate solutions applied around citrus, grapes, and ornamentals.

Ezekiel described a laboratory method of preliminary comparison of soil disinfectants in soil chambers. The disinfectants are mixed with moist soil, placed in jars with inoculum in the center of some jars and next to the glass walls of other jars, and the extent of growth from the inoculum is then noted.

King prevented the germination of sclerotia by moistening them 45 minutes in 1 per cent Formalin solution. Neal reported that sclerotia immersed for 25 minutes in 0.5 per cent solutions of various disinfectants were still viable; Semesan reduced the viability to 13 per cent; while immersion in a 1:1000 solution of bichloride of mercury for the same time reduced the viability to 27 per cent. After exposure of sclerotia for 50 minutes to 0.5 per cent solutions of copper sulphate and chlorophenol-nitrophenol-mercury solutions, the viability was still 60 per cent and 87 per cent, respectively.

Resistant varieties.—Rea, Dana, and Dunlavy reported that, during the last three years, 11,000 varieties and strains of cotton have been tested at the Blackland Substation for resistance to root rot, and all have been eliminated as susceptible except 41 strains which are still under test. Bach tested 104 varieties of grapes and 12 rootstocks over a period of three years. The Champenel, Mustang, and Black Spanish appear resistant; and *Vitis Champini* offers promise as a rootstock. Of the citrus tested, the Sour Orange rootstock appears highly resistant; the Cleopatra orange and *Citrus trifoliata* are very susceptible; and Rusk Citrange, Thomasville Citrangequat, and Ichan Lemon have also died from the disease.

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ATTEMPTS TO INDUCE "MIXOCHIMAERA" IN FUSARIUM MONILIFORME[1]

LEON H. LEONIAN

Brierly[2] suggests that one of the underlying causes of sectorial segregations among the colonies of bacteria and fungi may be traced to the possible phenomenon of "mixochimaera" whereby germ tubes and hyphae of different strains, varieties, and even species are supposed to fuse and give rise to mycelial threads containing cytoplasm and nuclei of distinct types. He suggests that "it is by no means impossible in view of the characteristic methods of hyphal growth and direct conidial abstriction, that new strains or types of fungi might arise. . . . " If one accepts the possibility of mixochimaera, one would be justified in assuming that a mixture and a partial or total fusion between the protoplasms of different strains or species would be capable of inducing some interesting and radical deviations from the parental types. With this idea in view, the writer undertook to demonstrate the possibility of synthesizing some new strains of *Fusarium moniliforme* by artificially associating distinctly different strains in petri-dish cultures.

ORGANISMS AND TECHNIQUE

A single-spore culture of a strain of *F. moniliforme* with which the writer has worked for the last two years has exhibited an extremely plastic nature by dissociating into more than twenty-five readily recognizable variants, not to mention scores of minor ones. In common with other dissociative types, some of these variants have remained fairly constant. Others fluctuate much and give rise either to complex colonies of newer variants or revert to the older types. Variant XII, being a stable form and having shown no marked deviation from type during the course of a very intensive cultural study, was selected as one of the experimental organisms. When grown on a nutrient agar consisting of 5 grams of peptone, 20 grams of dextrose, 1 gram each of dihydrogen potassium phosphate and magnesium sulphate, 20 grams of agar agar, and 1,000 cc. of distilled water, this organism forms a bright purple colony with scanty or no aerial hyphae. The second experimental organism was selected not from the foregoing variants but from another group of variants, the original of which was isolated from a totally different source. When grown on the same nutrient

[1] Approved by the director of West Virginia Agricultural Experiment Station as Scientific Paper No. 87.

[2] Brierly, William B. Variation in fungi and bacteria, Proc. Int. Cong. Pl. Sci. 1926, 2: 1629–1654. 1929.

agar, this strain forms an orange-color colony with rather short but abundant aerial hyphae of an orange hue. During two years, when this organism was kept under constant observation, it demonstrated a remarkable constancy of behavior. The purple organism has been termed type A and the orange form type B.

All cultural work was done with petri dishes, as test-tube cultures are highly unsatisfactory and often misleading in a study of sectorial behavior. All plates were 15 mm. deep and 100 mm. in diameter and contained 15 cc. of the nutrient agar described above. Hundreds of such plates were prepared and inoculated with types A and B. In some, the hyphae of the two types were planted together; in others, spore suspensions of the two were deposited in the center of the plate. In still others, agar cultures of the two types were ground together into a paste, then a portion of the paste was planted in the dish.

RESULTS

In nearly every case a complex colony consisting of mixtures of types A and B was the result. The two types were differentiated either at the very beginning of the young colony, where the purple and the orange growth sharply separated into sectors, or an apparently pure colony of either A or B type developed. However, after assuming a diameter of 30–40 millimeters it gave rise to a sector of the other type. Just how a purple mycelium was masked by an orange colony or an orange mycelium masked by a purple one and just how it broke loose to form a sector consisting of a pure culture of only one type are not clear.

Mixed plantings often showed another behavior: type A, of slower growth habit, was sometimes left behind by the orange form, thus appearing as a purple blotch in the center of the plate. Transfers made from the outermost edge of the orange colony always gave rise to pure orange colonies. In no case did the masking effect of one or the other type extend beyond the young colony. By the time the outermost growth reached the edges of the culture dish, it was found to consist of either one or the other type only; it seems that the mixed growth cannot continue indefinitely. Sooner or later the two organisms must separate.

Hundreds of cultures invariably showed this to be the case. Even when some of the most outstanding of the 25 variants were associated together in various combinations, again there was a sharp sectorial differentiation despite the fact that all of these 25 originally came from the same single spore. The same thing was found to be true when type B was associated with other species of Fusarium, such as *F. culmorum, F. herbarum gibberalloides,* etc.

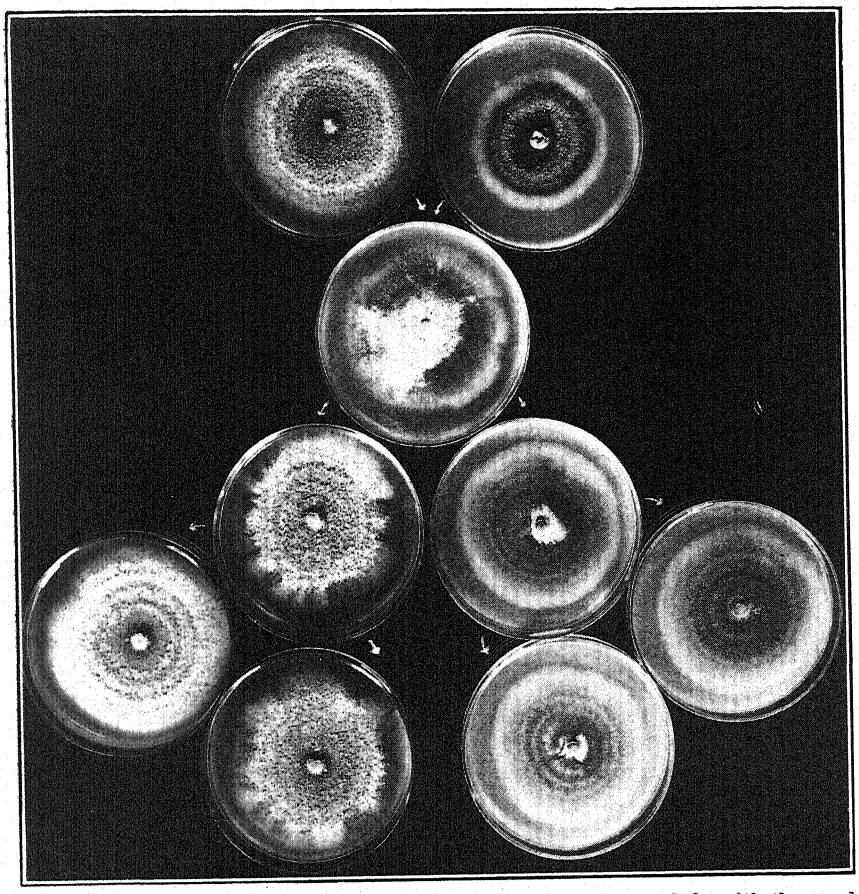


FIG. 1. Association of type A, upper right, and type B, upper left, with the resulting changes for three generations, as indicated by arrows. In the first-generation culture, second row, type A has been completely masked and suppressed by the more rapidly growing colony of type B.

In hundreds of cultures in which types A and B were associated, a new type of growth was observed at four different times. This was immediately isolated. This new type, described here as type C, resembled neither type A nor B but seemed to be an intermediate between the two. The young colonies were orange in color, thus resembling type B, but possessed no aerial mycelium, a characteristic of type A. As the colony grew older, it changed into a burnt orange, then purplish orange, and then nearly pure purple, not easily distinguished from type A. Upon growing still older, an orange-color aerial mycelium, exactly like that of type B, began to form all over the surface of the colony. Transfers made from this orange

mycelium, however, gave rise to type C colonies, only. The submerged mycelium of type C was found to be tenacious, yielding to the needle with difficulty; this holds true in case of type A, whereas type B has no tenacity and yields to the needle with great ease.

Young colonies of type A form macroconidia in great abundance and microconidia in comparatively scant numbers. Type B forms microconidia in great abundance and very few macroconidia after the colony is quite old. Very young colonies of type C form an abundance of microconidia and a few macroconidia, but, as they grow older, macroconidia appear in great numbers. One hundred single-spore isolations were made from this type, and in every case a pure colony of type C was formed. Circumstantial evidence thus pointed to a possible fusion between types A and B, giving rise to an intermediate form, type C. The only alternative was a possible dissociation of either type A or B and the formation of the new type.

While the remarkably intermediate habit of the new type at first seemed to exclude such a possibility, yet the writer's faith in a possible mixochimaera began to wane when the behavior of these strains on a different nutrient medium was observed. This medium consisted of the following ingredients: Dry malt extract 10 grams, dextrose 10 grams, potassium nitrate 2 grams, 1 gram each of dihydrogen potassium phosphate and magnesium sulphate, 20 grams of agar agar, and 1,000 cc. of distilled water.

On this agar type A was almost colorless, showing only very faint zones of lavender. Type B was orange, as usual, but type C was a deep blue green. Whence came this color? A mixture of orange and purple does not give such a shade. Since a mere physical mixture of the protoplasms, of nuclei or of fractions of nuclei of types A and B could not yield this blue green shade there could be only two explanations for such a behavior: First, that either type A or B dissociated to give rise to type C and, second, that there was an actual fusion and recombination of nuclear materials of A and B. Such an explanation could rest only upon the bold and unsupported hypothesis that a sort of amorphous sexuality was operative in these fungi.

Although the appearance of this blue green pigment rendered the possibility of mixochimaera rather remote, it was deemed advisable, nevertheless, to attack the problem with the hypothesis of mixochimaera as a basis. It was reasoned that if type C represented a more or less mechanical mixture of types A and B, a differential growth factor would serve to suppress or eliminate one of the two types and allow one of them to grow. Such oxidizing or toxic agents as potassium permanganate, potassium dichromate,

silver nitrate, mercuric chloride, and Semesan were employed to split the possible mixochimaeral union. The three types of organisms were grown in a nutrient solution consisting of 10 grams each of dry malt extract and dextrose, 1 gram each of dihydrogen potassium phosphate and magnesium sulphate, 2 grams of potassium nitrate, and 1,000 cc. of distilled water. Five cc. of this solution was poured in each test tube, sterilized, and inoculated.

After seven days' incubation at 25° C. a good mycelial mat was formed on the surface of the solution. These mats were removed and washed in three changes of sterile distilled water and transferred to a series of solutions consisting of 0.5 per cent of potassium permanganate, 0.075 per cent of Semesan, and 0.02 per cent each of silver nitrate, potassium dichromate, and bichloride of mercury. An hour later they were transferred to another series of corresponding solutions with 5 cc. of the solution in each glass capsule of 25 cc. capacity. Such a double transfer served to reduce the amount of distilled water held by the mycelial mat to a negligible quantity. These cultures were set aside and examined from day to day.

Rather unexpected results followed: The potassium permanganate solution was reduced by all three types within less than a day, and new hyphae appeared and grew into the solution. Growth took place in all other solutions as well, new hyphae not only appearing over the surface of the mycelial mat but growing out into the solution of pure bichloride of mercury and similar toxic substances of relatively high concentration. The work was repeated a number of times with similar results, except that type A sometimes failed to grow even after being transferred back to a fresh supply of the nutrient solution. However, since this behavior was not constant, no reliance could be placed on the effect of differential growth on the splitting of the hypothetical mixochimaera.

In order to see if such drastic treatment exerted any effect on the characteristics of the three types, transfers of the new growths were made from these toxic solutions to plates of nutrient agar. When colonies began to form, type A retained its characteristic in all of the cultures, while some of the cultures of type B split into a complex colony consisting of types B and C, and type C also formed a complex colony consisting of types B and C. These results eliminated any possibility of mixochimaeral phenomenon and conclusively demonstrated that type C was a dissociant of type B.

At this stage of the work it seemed that the effect of oxidizing agents or toxic substances enhanced dissociation. However, the writer always has maintained that, aside from the so-called unfavorable environmental conditions and stimuli of various types, other factors also may be responsible for the appearance of dissociative phases in the cyclogeny of fungi, such as

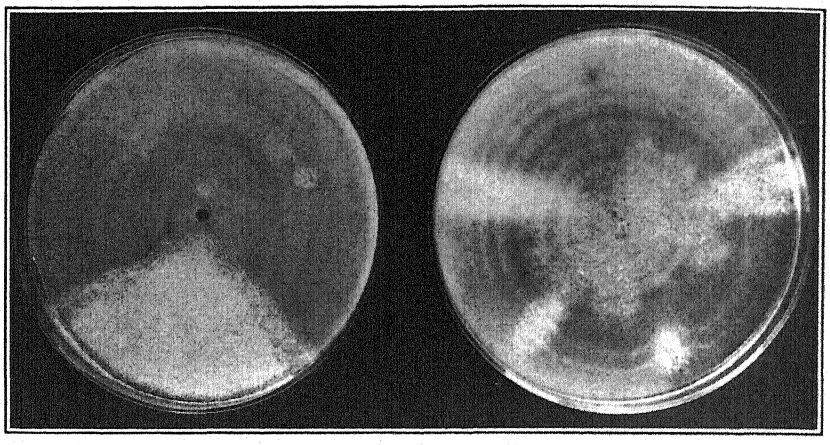


FIG. 2. Type C dissociating into type B (left); type B dissociating into type C (right).

the masking effect of different food substances, periodicity in the life cycle of organisms, and also chance and the personal-equation factors.

If one is doing an intensive cultural work with a given organism and is persistently watching for sectorial differentiations in growing colonies, one is more apt to detect and isolate dissociative phases. Because the writer assumed, to begin with, that type C resulted from the association of types A and B, his attention was centered upon an intensive study of the association of these two types. Had he made as many cultures of type B alone, he would have obtained type C as frequently and maybe more so.

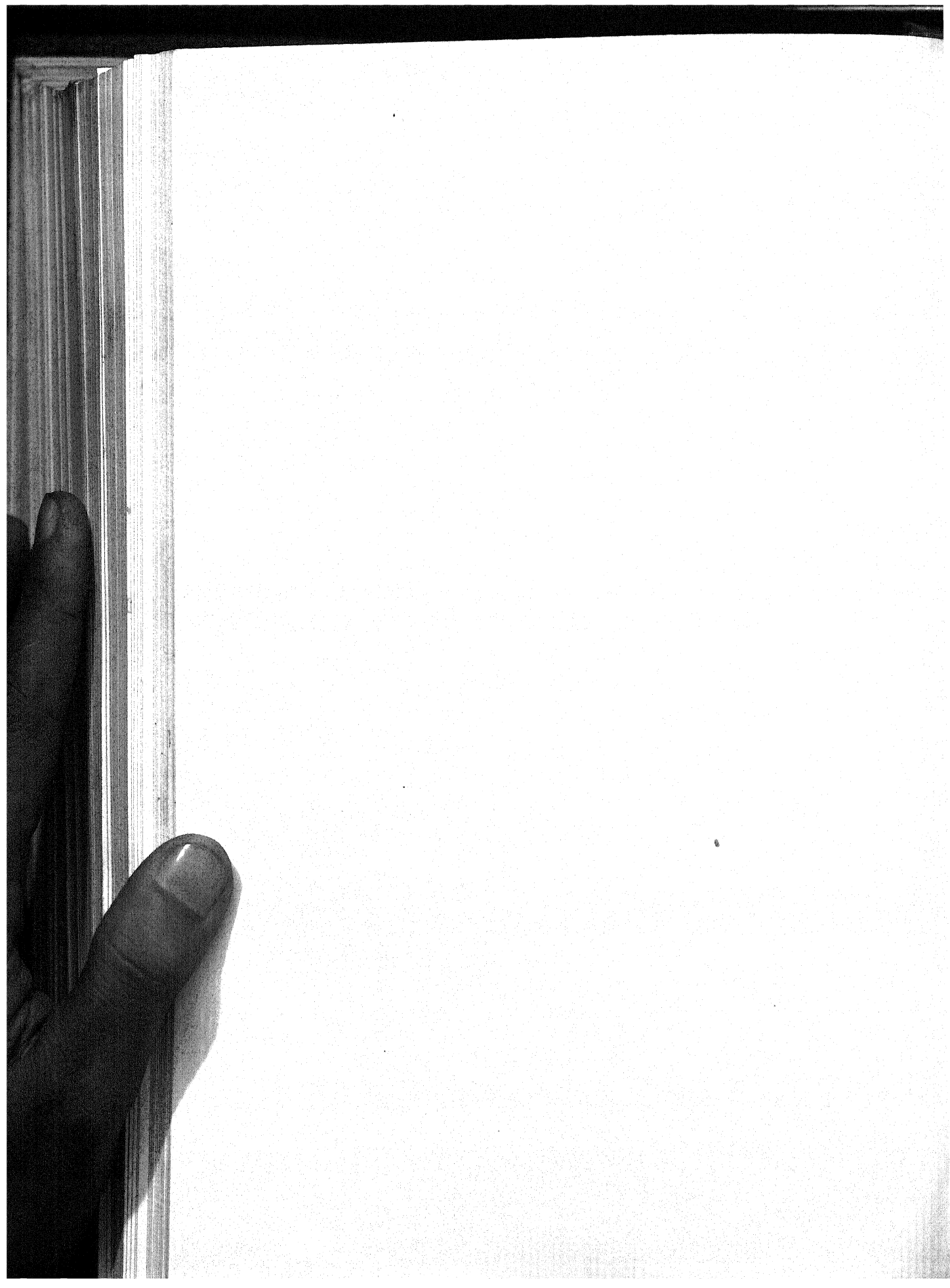
In order to demonstrate this, a large number of transfers were made from the original culture of type B not only to the standard agar used in this work but to a number of others as well. Within a week a number of complex colonies consisting of types B and C resulted on all of the agars. This is by no means an unusual behavior, as similar instances of periodicity have been observed by the writer both in Fusarium and Phytophthora species. While it is not possible sharply or even approximately to delimit the chance factor from the periodicity factor, it is safe to assume that both are present and active. Intensive cultural work extending over a long period of time is necessary for the proper unraveling of all possible dissociative phases of a given organism.

CONCLUSIONS

It is concluded that, in so far as the organisms and the conditions described in this paper are concerned, mixochimaera is not a factor in dissociation phenomena. A mere mixture of protoplasms cannot give rise to new characteristics, unless there be a sexual phenomenon involved, be-

cause each nucleus will behave as a unit and will develop its own characteristics. Wherever there is a sexual attraction and fusion, sexually formed reproductive bodies result and differentiate this type of behavior from dissociation phenomena. If one were to subscribe to the possibility of the occurrence of mixochimaera, one would then be prepared to witness all sorts of bizarre combinations. An intensive study of dissociants reveals the fact that, despite all deviations from the "normal" orbit of a given species, all of its dissociants retain a remarkable continuity of kinship. Their departures are all normal variations, appearing as "abnormal" or "new" to us because of our ignorance concerning the potentialities of the species. Hyphal fusions may occur between different strains, species, and even genera, but, if anything like mixochimaera could result and if more intimate fusions could follow, taxonomy would be infinitely more chaotic than it is to-day.

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TO WHAT EXTENT IS "SPRAY BURN" OF APPLE FRUIT CAUSED BY THE FREEZING OF THE FLOWERS?

L. H. MacDaniels and A. J. Heinicke

On May 14, 1928, in the Cornell University orchard at Ithaca, New York, the temperature fell to 24 degrees F. for a short time during the night. On this date most apple varieties were in the early stages of bloom with about half of the blossoms open. In examining the closed flowers to see if they had been seriously damaged, the petals were pulled off to expose the pistils. In many cases in trying to remove the petals the outer layer or layers of cells on the entire ovary and upper part of the pedicel slipped off, adhering to the perianth. A considerable number of varieties were examined and this condition was found in practically all. The styles of many of the flowers were reddish brown but the stigmas and the young ovules were apparently uninjured. It seemed as if sufficient injury had taken place almost to eliminate the crop. Contrary to expectations, however, good crops were harvested with most varieties.

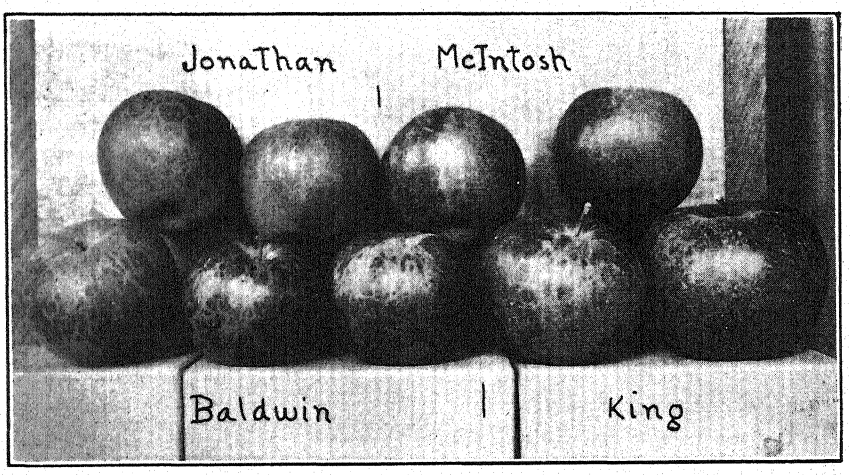


Fig. 1. Russeting of apples apparently caused by low temperature in the Cornell University orchard at Ithaca, N. Y., 1928.

During the summer and fall of 1928, it was observed that the fruit of nearly all varieties showed conspicuous russeting. The fruits shown in figure 1 illustrate the nature of this injury on the varieties Baldwin, Tompkins King, McIntosh, and Jonathan. During this season, russeting of a similar appearance was general throughout New York, particularly in

the Hudson Valley and in New England, it being much severer than usual. In most instances, growers attributed the injury to "spray burn." Although this may have been a contributing cause, it could not have been the only cause, for the injury was observed in orchards that had not been sprayed throughout the season. Further, nearly all varieties showed the injury this season. This was very unusual, as russeting from sprays is ordinarily confined to susceptible varieties, such as Baldwin and Ben Davis. It would, therefore, seem logical to suppose that the injury had been caused by the unusual cold of May 14.

In the spring of 1929 the temperature in the Cornell University orchard dropped to 29° F. on May 10 before any blossoms were open. On May 17, during the early stages of bloom, it fell to 31° F. Although some blossoms showed a little slipping of the epidermis, the injury was not frequent. During the season some netted russeting appeared in this orchard on Winter Banana and Baldwin, but not on other varieties. This lack of injury from freezing was to be expected, as 29° F. is known not to be a critical killing temperature for apple tissues. The 1929 observations thus tend to confirm the idea that the injury in 1928 was due to the low temperature prevalent while the trees were in blossom.

Spray burn often comes about through the application of spray material under unfavorable conditions. It is well known that lime sulphur applied at high temperatures is likely to cause the russeting characteristic of this injury (Heald,[1] p. 218) and that Bordeaux mixture applied at lower temperatures may have the same result (Heald,[1] p. 211).

Under some conditions, however, russeting is known to be produced by late spring freezes or frosts occurring after petal fall, entirely apart from the common forms of spray injury (Heald,[1] p. 151). Most growers are familiar with the frost bands or rings in which a band of russet extends around the fruit either at the calyx end or sometimes midway between calyx and base. This frost ring may be bulged out by the hypertrophy of the cells in that region or, again, the ring may be somewhat sunken. Not infrequently, however, russeting caused by freezing injury is much more diffuse and may cover the entire apple with a network of russet lines, as indicated in figure 1. In the season of 1928 this type of injury was clearly associated with freezing before the flowers opened. Such injury is difficult or impossible to distinguish from the real spray burn and this has led to a misunderstanding of the spray burn problem, in general.

It is entirely conceivable that russeting due to freezing may be caused by cold occurring even earlier than that reported in 1928 or shortly after the buds begin to swell. It is of interest also in this connection that hardy

[1] Heald, F. D. Manual of Plant Diseases. McGraw-Hill, New York. 1926.

varieties, such as Northern Spy and McIntosh, are less susceptible to russeting at Ithaca than are the recognizedly more tender sorts, such as Baldwin and Ben Davis.

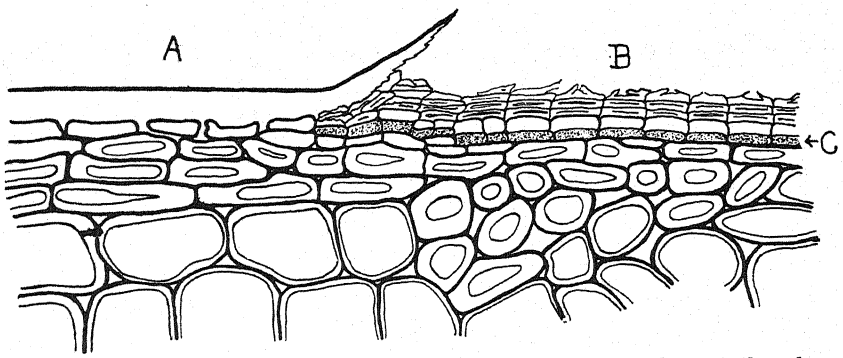


Fig. 2. Section of apple fruit taken at right angles to the surface at the edge of the russet area.

The histological nature of this injury is shown in figure 2, which represents a section through the edge of the russeted area on a fruit of Tompkins County King. At A is shown the normal cuticle and epidermis with normal fruit structure underneath. At B the normal epidermis has been destroyed and the surface covered with cork formed by the cork cambium C. It is probable that the lesion is due to the lethal freezing of epidermal cells or those near the epidermis and that the wound has been corked over by the activity of a periderm layer formed in living cells beneath the injured tissue. It will be noted that the fruit cells underneath the periderm are somewhat abnormal and apparently have arisen from the proliferation of the parenchyma beneath the injury.

The question as to why the injury occurred in a rather localized manner in some fruits and diffusely on others is difficult to explain. With a diffuse or net-like lesion it is probable that there were slight differences in the killing temperatures of the cells of the epidermis or other tissues affected and that the temperature dropped to a critical point where some cells survived and others did not. Where the russeting is localized in a continuous patch, it may be that the cells in that area were frozen to death because exposure to wind actually cooled them to a point lower than that of the uninjured parts of the epidermis or other uninjured and adjacent tissues. There is little evidence that sunlight on the frozen tissue would aggravate the injury either by more rapid thawing or by desiccation (Chandler).[2]

[2] Chandler, W. H. Fruit Growing, Chapter 21. Houghton Mifflin, New York. 1925.

It is not improbable that russeting from frost injury is aggravated or increased by the application of spray materials, which penetrate and injure the already damaged tissues more than would be possible if the frost injury were not present. This, however, needs further confirmation.

There are observations in the Cornell orchard to indicate that the nature of the russeting may depend on the stage of development of the young fruit or flowers at the time of the freeze. Where the freeze occurs before the flowers of the blossoming clusters separate, the injury has been observed to be confined to the definite sectors of the fruit, touching each other at that time. Contact of the young ovaries of the blossoms with each other or adjacent leaves may also have something to do with the frost rings that are frequently formed.

Certainly, the whole matter of the relation of freezing to russeting is not well understood. The purpose of this paper is merely to put on record observations that may corroborate the experience of others and lead to a better understanding of the whole problem, particularly as it concerns russeting caused by freezing at the time of blossoming or before, rather than after, the petals fall.

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WHEAT TAKE-ALL SYMPTOMS COMPARED WITH INJURIES CAUSED BY CHINCH BUGS[1]

HURLEY FELLOWS[2]

INTRODUCTION

In that portion of the United States where both take-all (*Ophiobolus graminis* Sacc.) and chinch bugs (*Blissus leucopterus*) are present, there is doubt sometimes as to which has caused the evident injury in some wheat fields. Both pathologists and entomologists have experienced difficulty in distinguishing between these maladies. It is much more difficult to tell the

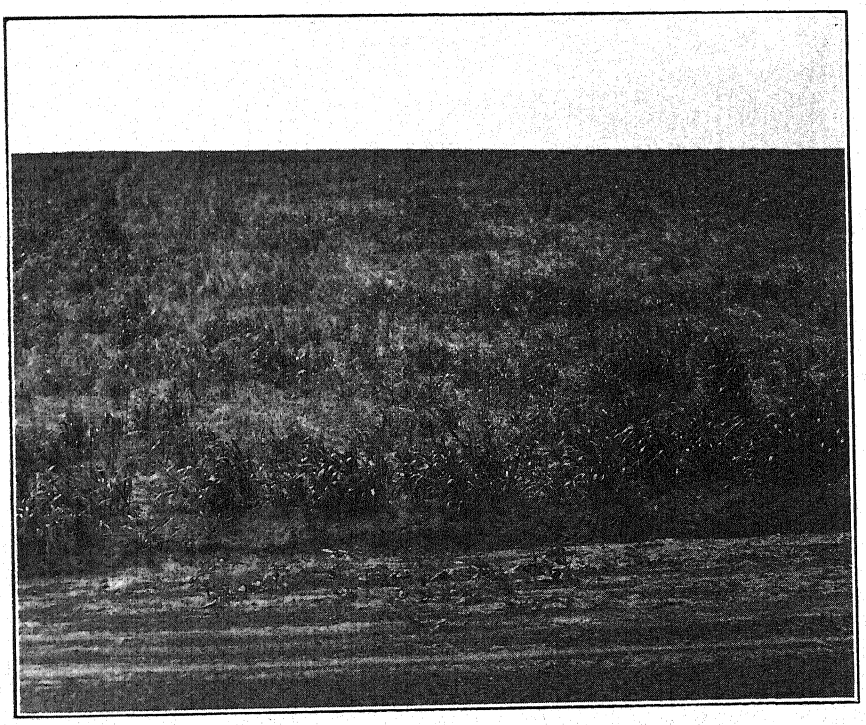


FIG. 1. An experimental plot where most of the wheat plants had been killed by take-all and chinch bugs. In such cases detailed examinations of individual plants are necessary for proper diagnoses.

[1] Contribution No. 296 from the Department of Botany and Plant Pathology, Kansas State Agricultural College, in cooperation with the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Associate Pathologist, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

difference when both take-all and chinch bugs (Fig. 1) are present in the same wheat field. The present paper attempts to make clear the similarities and differences.

METHOD

Chinch bugs were caged with wheat plants growing in pots containing soil known to be free from the fungus causing take-all. The chinch bugs were allowed to feed until the plants were killed. This made it possible to observe the chinch-bug damage at all stages. Wheat plants were grown also in pots containing soil infected with *Ophiobolus graminis*.

In the field the chinch bugs were induced to congregate in the immediate vicinity of a wheat plot by spreading thin layers of straw for protection. In the spring, after the chinch bugs started migrating, cold periods made them seek the straw in large numbers. When warm weather came, they were present in multitudes to feed on the wheat plants.

COMPARISON OF SYMPTOMS

The similarity between the symptoms of the injuries caused by the two parasites is mostly in the general behavior of the wheat plants attacked. Wilting, browning, and final death of the leaves occur in both cases. This no doubt is due to the somewhat similar action of the parasites. The writer has shown that *Ophiobolus graminis* disintegrates both the phloem and the conjunctive tissue[3] of the wheat plant. Painter has pointed out that in corn, milo, and sorghum the chinch bug feeds chiefly on the contents of the phloem. He has shown also that the phloem often is plugged with special deposits of plant materials[4] and that these form a sheath around the wounds produced by the stylets of the chinch bugs. However, chinch bugs feed chiefly on the phloem in the leaf sheaths; whereas, *O. graminis* invades primary and secondary roots, subcoronal internode, crown, leaf sheath, and culm tissues. In the case of take-all, the occurrence of the fungus on other tissues as well as on the leaf sheaths is good proof of the cause of the injury.

Both take-all and chinch-bug injuries often occur in spots in the field. Chinch bugs are gregarious and apparently prefer to feed in more or less open spots where stands are thin. Take-all spots, however, may and often do occur in the interior portion of fields with very heavy stands. Furthermore, take-all spots have a very distinct margin: that is, there is a sudden transition from the short diseased plants in the spot to the surrounding healthy plants of normal height. Chinch bugs cause a more gradual transition at the margin of spots.

[3] Fellows, Hurley. Some chemical and morphological phenomena attending infection of the wheat plant by *Ophiobolus graminis*. Jour. Agr. Res. 37: 647–661. 1928.

[4] Painter, R. H. Notes on the injury to plant cells by chinch bug feeding. Ann. of Ent. Soc. America 21: 232–242. 1928.

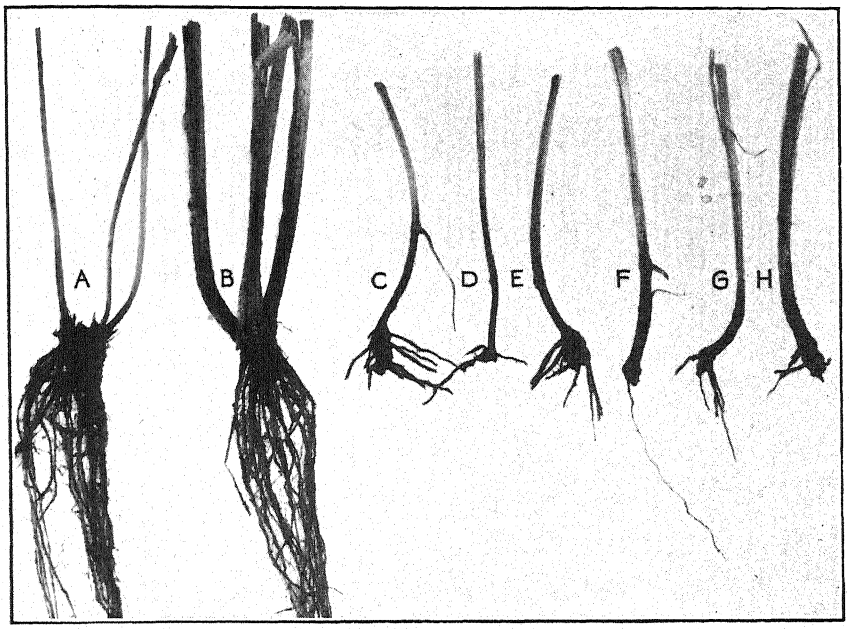
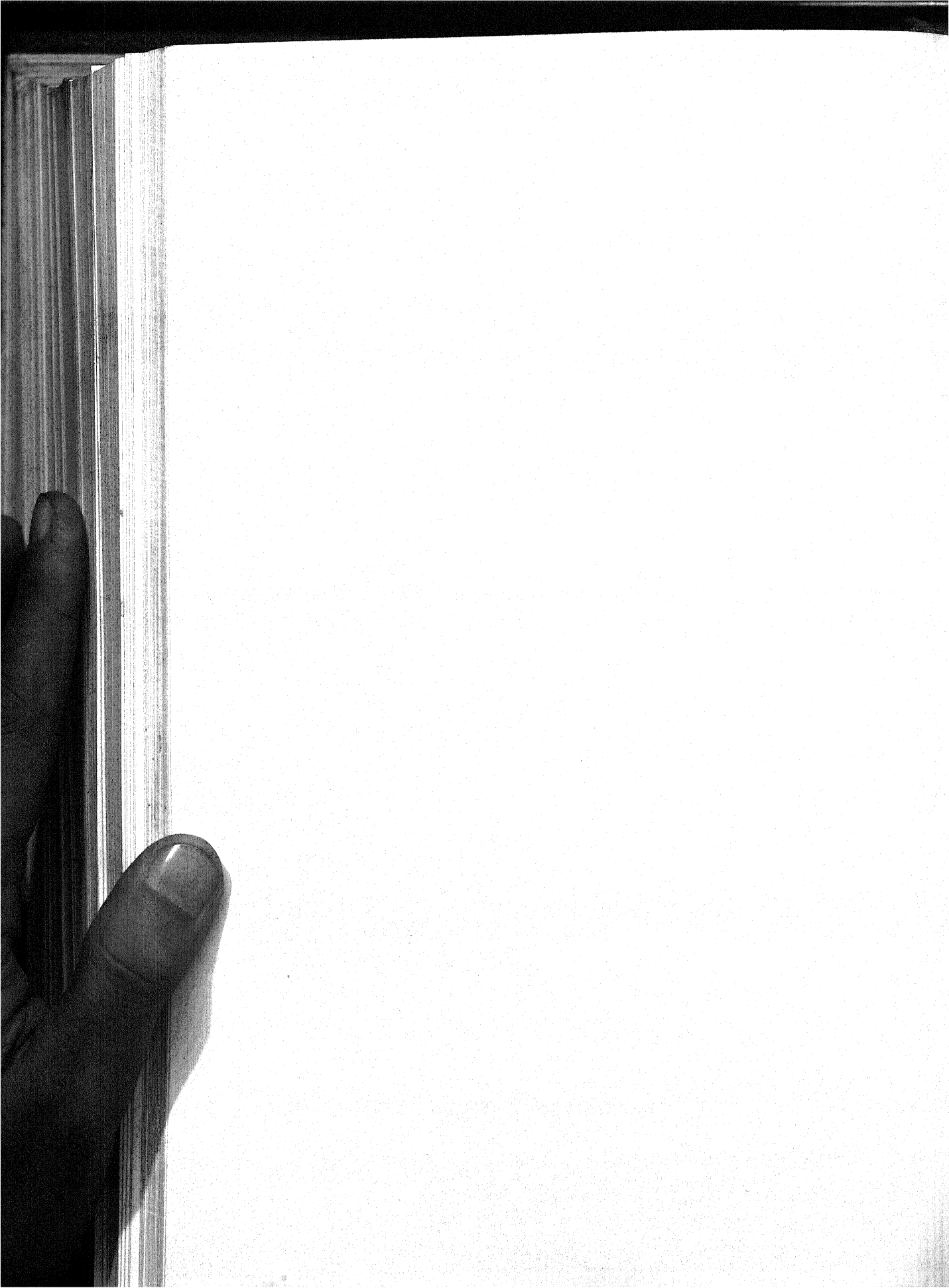


FIG. 2. Bases of wheat culms. A and B, killed by chinch bugs; C–H, killed by take-all. A, C, D, and E have the lower leaf sheaths removed, whereas the others have not.

For a reliable determination of the agent responsible for the injury, entire individual plants should be examined. Wheat plants attacked by chinch bugs have a good root system free of lesions. They also tiller well. If any discoloration occurs at the bases of the culms, it is of a brownish tinge; but it is only in the advanced stages of injury that any discolorations appear. On the other hand, the take-all fungus invades the crown, hinders the original formation of roots, and causes those that are formed to become black and brittle. The bases of the culms become black and shining. Then, too, there often is a mycelial plate on the surface of the lower culm, never present on plants injured only by chinch bugs (Fig. 2). It also has been shown by various authors that tillering is materially reduced following invasion by *Ophiobolus graminis*.

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A WASHING DEVICE FOR ISOLATION WORK WITH PLANT MATERIAL

P. M. SIMMONDS

An efficient device for washing pieces of plant tissue and seeds from which isolations are to be made has been devised and used, with quite satisfactory results, by the Dominion Plant Pathological Laboratory, Saskatoon, for the past two years. The isolations were chiefly for fungi. It has proved especially useful in making isolations from cereal grains. The usual practice when making such isolations has been to surface sterilize the plant fragments with solutions of bichloride of mercury, silver nitrate, or other chemical. With the device herein described, the pieces may be washed thoroughly with sterile water and plated immediately. Most workers have noticed that where poisons are used the growth from the isolation pieces is greatly retarded. Furthermore, it is just about impossible to use a poison on such delicate tissues as fine rootlets, etc., for, in such cases, the desired fungus may be killed outright. By washing with sterile water these disadvantages disappear, growth is rapid, and isolations are more readily obtained.

In the writer's studies many isolations were made from the fine rootlets of cereals. In using this device an increase in isolations of 100 per cent over the bichloride of mercury method was often obtained. Almost invariably there was an increase in fungi secured as well as more rapid growth. At times, when isolations were attempted from material that was not fresh or that was coated by much foreign matter, black and blue molds appeared. Black molds were most troublesome because of their rapid growth. In such cases this difficulty was largely overcome by promptly making subcultures. Washing a large number of pieces by means of a wash bottle or by rinsing becomes very tedious and laborious. The apparatus here described can wash a large number of pieces thoroughly and rapidly.

The general set-up of the apparatus is indicated in figure 1. A two litre flask, A, is fitted with a glass tube which passes through a cotton plug to connect at E. When disconnected the end of this tube may be protected by a cotton plug either wrapped or tied for security. A flask so fitted and filled with distilled water can be sterilized readily. Several such reserve flasks are kept on hand. B is a funnel plugged with absorbent cotton and serves as an air filter. A small, wide-mouth 100 c.c. flask, C, fitted through a rubber stopper with two aeration tubes, as shown, serves as washer, while flask D, connected with an ordinary filter pump at P, collects the water drawn through the apparatus. Flask C is held in place by means of swivel

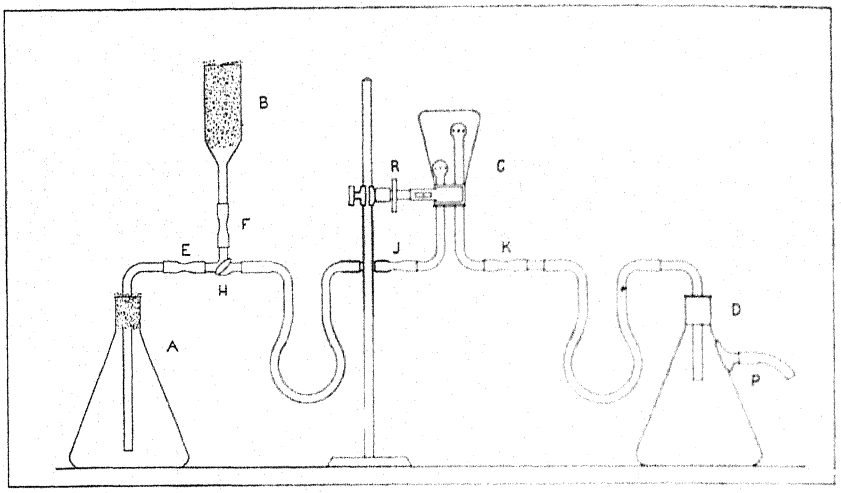


FIG. 1. A washing device for use in making isolations of fungi from plant material. A description is given in the text. (Drawn by G. Verbeke.)

clamp R and so can be inverted readily. A three-way stopcock, H, is used to control the water and air intake. Pinchcocks are used at J and K.

To use the washer, flask C is disconnected at J and K and taken from the clamp. The stopper is removed and the pieces of tissue or seeds to be washed are placed therein, after which the flask is replaced and connected. Pinchcocks at J and K are then released, the pump is started, and the stopcock at H is adjusted so as to allow water to be drawn into C. When the flask is full, H is again adjusted so as to close off the water intake but to permit air to enter through B. This will subject the material in flask C to thorough agitation. After this has continued for a minute or so and the water has decreased slightly as some is sucked over, flask C is inverted. The water is then drawn off into flask D through the aeration tube which extends to the bottom of flask C. The swivel clamp permits this movement without difficulty. After the water is drawn off the flask is returned to its former position, the air is closed off, and the water allowed to enter as before. Then the water is stopped and air introduced for agitation and so on. This rinsing or washing may be continued as many times as desirable. Experience quickly teaches when the process is complete. At the end of the process enough water is drawn into C to float or cover the material, then pinchcock K is closed, the pump is turned off, and pinchcock J is closed. This procedure prevents any back pressure. Flask C is now disconnected at J and K, using these pinchcocks and rubber tubing to close the ends of the aeration tubes. The material may then be removed with sterile forceps under ordinary aseptic conditions. By having several

washer flasks fitted up one man can be plating while another is manipulating the apparatus. Thus a large number of pieces can be plated in a relatively short time.

It should be mentioned that when the washing is in progress flask C may need to be shaken at times to remove material that may lodge against the outlet aeration tube. The holes in these tubes are not always small enough to prevent very fine material from passing through; in such cases a fine-mesh copper wire screen can be sealed into the ends of ordinary glass tubes to replace the aeration tubes. Other adaptations may be made, depending upon the circumstances.

If a three-way stopcock is not available, an ordinary Y connection may be employed by the proper manipulation of pinchcocks at E and F. The material should be washed under the tap as cleanly as possible before cutting it up into the pieces from which isolations are to be made.

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SMUT RESISTANCE IN MILLET

R. H. Porter,[1] H. K. Chen, and T. F. Yu

In previous papers[2,3] the writers mentioned the seriousness of kernel smut of millet (*Ustilago crameri* (Körn)) in north China. In the summer of 1925, while inspecting some cooperative experimental plots in the provinces of Honan and Shantung, the senior writer made a number of head selections from a millet field near Wiehsien, Shantung, and at the same time he secured from Mr. E. J. Middleton, at Kaifeng, seed samples of a number of millet varieties and selections.

In the spring of 1926 an experiment was planned to test the reaction of these selections and varieties of millet to kernel smut. About 700 head selections and 57 other samples, some of which were varieties, were included in this first test. Seed of each sample was inoculated with smut spores by means of a tea strainer in which the seed and smut powder were mixed together. After the smut was strained through the sieve, the seed was placed in an envelope to be used later for planting.

The inoculated seed was measured out in approximately 3-gram samples and planted in rows 5 feet long with a foot between each row. Every tenth row was planted with inoculated seed of a common susceptible variety as a check. Unfortunately this seed used for the check was not viable, so that no readings could be made, but the percentage of smut was so high in most of the selections that there was no question about the effectiveness of the method of inoculation. Records on all of the rows were not preserved for 1926, because the smut infection ran as high as 70 per cent in some cases and these selections were discarded. The inoculum used in 1926 and in subsequent years was gathered from smutted heads in a field at Weihsien, Shantung, in 1925. All plantings were made on the experimental plots of the University of Nanking, Nanking, China.

In the succeeding years, some slight variation in the method of planting was made; for example, in 1927 and 1928, the rows were 8 feet and 16 feet long, respectively, and each plot was duplicated and planted in a different location. In 1929 only the most promising selections were included and each sample was replicated 10 times in rows 16 feet long. In addition, 19 of these selections were planted in the greenhouse in rows 1½ feet long. The

[1] At the time these investigations were initiated, the senior writer was Plant Pathologist at the University of Nanking, Nanking, China.

[2] Porter, R. H. Seed disinfectants for the control of kernel smut of foxtail millet. Proc. 3rd Pan-Pac. Sci. Cong., Tokio. Pp. 2103–2107. 1926.

[3] Porter, R. H., T. F. Yu, and H. K. Chen. The effect of seed disinfectants on smut and on yield of millet. Phytopath. 18: 911–919. 1928.

planting dates were: June 23, 1926; June 22, 1927; June 20, 1928; and June 14, 1929. The results of the four-year test are presented in table 1, in which both resistant and susceptible strains are listed for purposes of comparison.

TABLE 1.—*Reaction of varieties and strains of millet to kernel smut caused by Ustilago crameri*

Nanking No.	Average per cent smut					Source No.
	1926	1927	1928	1929		
	Field	Field	Field	Field	Green-house	
2	0	0	0	.....	.....	Shansi 1
3	0	8.2	45.5	.....	.....	Kaifeng 3
6	Trace	0.9	1.8	1.7	4.8	" 16
9	Trace	0.9	1.2	2.7	10.0	" 20
12	0	0	0	0.3	0	" 23
13	0	0	1.4	.....	.....	" 24
16	0	0	0	7.6	0	" 27
17	0	0	0	1.1	0	" 28
18	0	0	0	0.1	0	" 29
19	0	0	0.4	0	0	" 30
20	.....	0	27.4	.....	.....	" 31a
26	0	0	0	0.1	0	" 31b
28	Trace	0	0.9	2.7	0	" 41
29	Trace	0.3	2.0	1.5	0	" 43
30	0	0	2.1	0.1	0	" 44
31	0	0	0	0.3	0	" 45
35	0	0.2	0	0.2	0	" 49
37	0	0	0	1.7	0	" 51
38	Trace	0.2	0	1.8	.....	" 52
39	0	2.7	9.7	.....	.....	" 53
42	.....	22.7	26.1	.....	.....	" 56
45	0	0	4.2	.....	.....	" 59
47	0	0	0	0	0	" 61a
48	.....	0	7.6	.....	.....	" 61b
53	.....	3.6	16.9	.....	.....	" 72
56	0	0	0	1.2	0	" 80
57	0	0	0	0	0	" 81
58	.....	17.7	50.8	.....	.....	" 85
H–28	.....	.....	1.3	0.4	0	Peking-Sel.
H–278	.....	.....	42.8	.....	.....	" "
Inoculated checks	Seed not viable	7.1	33.2	35.1	51.0	

The results in the above table show that about 20 of the millet selections are highly resistant to smut; in fact, two showed no smut in any of the tests and ten others showed only a trace in one or two years out of four. Apparently, there are strains or varieties of millet resistant to kernel smut, a fact that may prove of great value in the control of the disease.

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PHYTOPATHOLOGICAL NOTES

Two new diseases of cultivated mushrooms.—During the past year commercial mushroom growers have suffered considerable loss and inconvenience from two diseases that have not been described in the literature. The symptoms and etiology of these diseases are quite different and they are apparently in no way associated. The outstanding characteristic of one of them is the malformation of individual mushrooms by the production of numerous intumescences and patches of gills over the surface of the pileus. These hypertrophied growths often resemble the rose-comb of poultry and have suggested the name *rose-comb disease.* The other disease is characterized by a reduction in the yield of mushrooms, especially during the latter half of the crop, coincident with the appearance over the surface of the soil of wrinkled discoid patches of fungus tissue and, throughout the compost, of cerebriform subglobose fungus bodies. These bodies are the ascocarps of an undescribed species referred to the genus Pseudobalsamia, and they suggest the name *truffle disease.*

For several years occasional specimens typical of the *rose-comb disease* have been submitted to the Office of Mycology and Disease Survey. Dur-

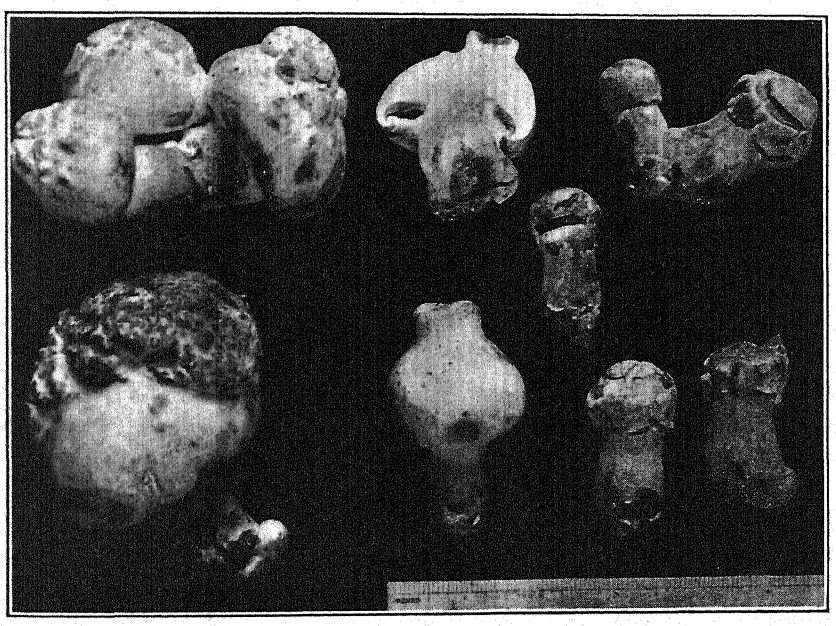


FIG. 1. *Rose-comb disease* of the "snow white" variety of mushrooms, received from a commercial grower in Chester County, Pennsylvania. Photographed by J. F. Brewer.

ing the past season the disease was unusually prevalent and complaints accompanied by specimens were received from New York, Pennsylvania, Ohio, and Missouri. As mentioned above, the principal symptoms are the production of wart-like intumescences, deep seams, and superfluous gills over the surface of the pileus (see Fig. 1). They are strongly suggestive of crown gall, and bacteria occasionally may be isolated from the hypertrophied tissue. But these organisms have not caused tumors upon reinoculation and do not resemble the crown-gall organism. Furthermore, a virulent culture of *B. tumefaciens*, loaned to me by Miss Nellie A. Brown, Office of Horticultural Crops and Diseases, was innocuous when injected into healthy mushrooms. The agencies that seem most likely to be the cause of the tumors are coal-oil fumes or the fumes or oxidation products of materials contained in mineral oil. In all of the cases I have been able to investigate the disease was correlated with the use of some form of mineral oil in the mushroom houses, and the discontinuance of this practice eliminated the trouble. In several cases the disease was correlated with the use of a proprietary insect spray containing pyrethrum and kerosene, in others with the excessive use of kerosene in fly traps and as a direct spray, and in one case with the application, to mushroom beds, of water containing lubricating oil that came from an expansion tank made from a discarded oil drum. Instances of this kind are clear-cut and apparently too numerous to be mere coincidences. On the other hand, it must be admitted that we have not yet determined the factors necessary for the consistent production of the symptoms under experimental conditions.

The *truffle disease* was first called to my attention in April, 1929, by a grower at Ashtabula, Ohio. Since then I have observed it in Minnesota, Pennsylvania, and New York and have received typical specimens from Colorado. Under certain conditions the disease may completely prevent the development of the latter half of the crop in an infested house. It is especially serious in slow-growing "runs." During the past season in New York, one grower alone suffered a loss from the disease of at least $100,000. After the first few "breaks" affected beds can be easily recognized by the development of fungus wefts and rudimentary mushrooms under the sideboards and sometimes over the casing soil and by the growth over the soil and in the compost of yellowish wrinkled fruiting bodies that are discoid on the surface and subglobose in the compost. The disks range in size from that of a dime to a half-dollar, while the subsurface fruiting bodies are from 3 to 30 millimeters in diameter. They are the ascocarps of an undescribed species of truffle which undoubtedly is the cause of the diseased condition of the beds. The species (to be described in a current number of Mycologia) is apparently most closely related to the genus Pseudobalsamia, but

it has smaller spores and differs in other respects from any known truffle. The ascocarps usually begin to appear scattered over the surface of the soil and in the compost about a week after the area inhabited by the fungus has ceased bearing mushrooms.

Very little is known regarding the source of the truffle, the factors affecting its accumulation in the compost, its relation to the cultivated mushroom, its persistence from one crop to another, and control measures. Circumstantial evidence suggests that it is a widely distributed soil organism which is inconspicuous until it enters the mushroom house and finds a favorable medium for extensive development in the bed. Whether it is a parasite or acts as a weed in the bed is problematic. In some cases it has seriously affected one or two crops in a mushroom establishment and, later, for no apparent reason, failed to develop in subsequent crops.—EDMUND B. LAMBERT, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

Cleaning up strawberry stock infested with root-gall nemas.—Observations for several years, in the strawberry breeding grounds at the U. S. Plant Field Station at Glenn Dale, Md., seemed to indicate that, with care, it was a comparatively easy matter to greatly improve any stock of strawberries infested with the root-knot nematode (*Caconema radicicola*). In 1928, several selections were so badly infested that they could not be distributed. These were planted on a relatively heavy soil free of root-gall nemas with the idea that runner plants would be free from the nema. In 1929, when runner plants were dug, they were, in nearly every instance, found clean. A more severe test was therefore made. Plants of a variety with the heaviest infestation yet observed (Fig. 1, A) were brought to the Station and planted on a nema-free clay loam. In the spring of 1930, the old plants showed some galls but far fewer than the set plants. The runner plants, however, were entirely clean (Fig. 1, C). The reason for the clean runner plants from the infested mother plants is the known fact that the nemas have difficulty in moving through heavy soils and were, therefore, unable to penetrate the clay soil to reach the roots of the young plants.

Strawberry-plant growers of necessity locate their nurseries on light soils because the plants can be dug in the spring when they could not be dug from heavy soils. Moreover, the root systems of plants from light soils are considered better. However, many plant growers can select small areas of relatively heavy soil to use in propagating a clean stock for their own planting. If a clay soil cannot be used, the cleanest plants from the edges of rows grown on fine sandy soil may be used to set a second bed in nema-

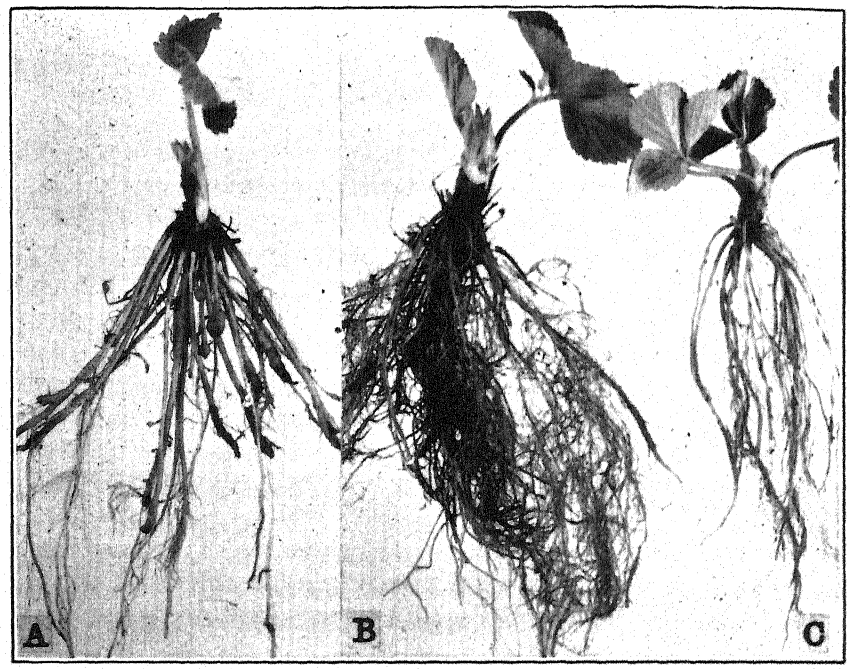


FIG. 1. The three plants shown are all of the same variety, U. S. D. A. No. 657, of strawberry. Plant A was grown at Willard, N. C., and shows the infestation there by the root-knot nematode. Plant B was a similarly infested plant but taken to Glenn Dale, Md., and grown in heavy soil for one year. A few infested roots can be seen, but the infestation is much less than when it was planted. Plant C is a runner plant grown from infested plants at Glenn Dale, Md., in heavy soil. No galls or indications of the nema were found on it. With care clean stocks can be propagated from infested stocks by growing on clay soils.

free soil and, with care, clean stocks can be obtained in two years.—GEORGE M. DARROW and GEORGE F. WALDO, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Carrot and parsley yellows transmitted by the six-spotted leafhopper, Cicadula sexnotata (Fall.).—A number of plant pathologists have called attention to a disease of carrots having most of the characteristic symptoms of yellows, but whether it was caused by the aster-yellows virus remained to be determined. In New York, Whetzel[1] reported a yellows disease of carrots ranging from a trace to 1 or 2 to 25 per cent infection in the Williamson area. Folsom[2] found apparently the same carrot disease

[1] Whetzel, H. H. Diseases of muck crops in New York. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. 13: 115–118. 1929.

[2] Folsom, D. Yellows. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. 13: 148–149. 1929.

as described by Whetzel, at Orono, Maine, and on the experimental farm in the southwestern part of the State. Zundel[3] reported the observation of yellows believed to be caused by the aster-yellows virus in carrots in Cumberland County, Pennsylvania.

During the spring and late summer of 1929 a survey was made of the yellows of plants grown on the ranch of the Morse Seed Company located in the Salinas Valley, California. It was observed during early June that the inner leaves of many varieties of carrots were yellow, but the plants were small and the symptoms were just beginning to develop. During late summer parsley and the white varieties of carrot showed some of the characteristic symptoms of yellows but, unfortunately, the varieties of yellow carrots had been pulled.

Experiments demonstrated that noninfective six-spotted leafhoppers, *Cicadula sexnotata*, after feeding on White Belgian, Short White carrots, and Hamburg parsley naturally infected with yellows, became infective and transmitted typical yellows to healthy asters and celery. Leaf hoppers, after feeding on the infected asters and celery, transmitted the disease back to the same varieties of healthy white carrots and parsley.

The same varieties of carrot and parsley grown from seeds were experimentally infected with yellows by infective leafhoppers. After symptoms of yellows developed, noninfective six-spotted leafhoppers feeding on the experimentally infected plants became infective and transmitted the disease to healthy asters and celery. The disease was transmitted from infected asters and celery back to healthy carrots and parsley. Yellows disease was also transmitted from infective carrots to healthy ones and similarly from parsley to parsley, carrot to parsley, and parsley to carrot. These experiments proved that the virus of carrot, parsley, celery, and aster yellows is identical.

Carrots and parsley infected with yellows showed a marked yellowing of the younger central leaves, while the older outer leaves were usually reddened. The younger central leaves were dwarfed and the petioles sometimes twisted; occasionally a dense growth of adventitious chlorotic shoots developed at the center of the crown. The roots were usually dwarfed, with bunched rootlets.—HENRY H. P. SEVERIN, California Agricultural Experiment Station, and United States Department of Agriculture, Bureau of Entomology, Berkeley, California.

[3] Zundel, G. L. Yellows (virus) on various plants. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. 13: 174. 1929.

Pear blight on Cotoneaster.—Species of Cotoneaster in the neighborhood of Riverside, California, are frequently infected with the pear-blight organism (*Bacillus amylovorus* (Burrill)). During the spring of 1929 a definite pathogene was isolated from a species of Cotoneaster. This was grown in pure culture and showed the characteristic growth of *B. amylovorus* in the media tested. Artificial inoculation on shoots of *Pyrus communis* (pear) and *Cotoneaster panosa* gave typical lesions. The organism was subsequently reisolated from these inoculations. So far as the writer knows, Cotoneaster has never before been tested as a host for *B. amylovorus*.—CLAYTON O. SMITH, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

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FRANKLIN SUMNER EARLE

PHYTOPATHOLOGY

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FRANKLIN SUMNER EARLE
1856—1929

C. L. SHEAR[1]

I am glad to accept the invitation to prepare a biographical sketch of our recently departed friend and colleague, as it affords an opportunity at the same time to pay a tribute to his high character, as well as to his scientific attainments as a mycologist, horticulturist, and agronomist.

Professor Earle passed away at his home in Herradura, Cuba, January 31, 1929, after an attack of bronchitis which developed into pneumonia. He was born at Dwight, Illinois, September 4, 1856. Very early in childhood he began to show much interest in plants and animals, and this was stimulated and encouraged by his mother, Melanie Tracy Earle. Being brought up on a large fruit farm, he had ample opportunity to become familiar with plant life in its varied aspects. His father, Parker Earle, was at this time one of the largest commercial fruit growers in that part of the country, and, although not a botanist, was deeply interested in plant life in its scientific as well as its cultural and commercial aspects. On account of his favorable environment, as well as inherited tendencies toward a love of Nature and a desire to become acquainted with her secrets, the boy soon developed a deep and lasting interest in plant life in general, as well as in its horticultural aspects. These two tendencies competed for control of his activities throughout his life, as will be shown by the various lines of work in which we find him engaged at different periods. At the age of sixteen he was prepared for college and entered the University of Illinois, but his services were so much in demand in connection with his father's fruit-growing operations that he spent only part of each school year at the University. After two fall and winter terms as a special student, we find him for the next four years assisting his father in his horticultural work. In 1878, he returned again to the University as a regular student in botany, studying fungi under Professor Burrill. He withdrew in March, 1879, to return to fruit growing.

His deep interest in botany, especially the fungi, which had been stimulated by his work with Professor Burrill, brought him back to the Univer-

[1] I wish to express my gratitude to Mrs. Earle, Mrs. Wm. T. Horne, sister of Prof. Earle, and to Mr. A. B. Seymour, for information not otherwise obtainable, and to Mrs. Earle for the accompanying portrait.

sity again in 1882 as a volunteer assistant, in order to pursue his favorite study and to help Professor Burrill in working up the Erysiphaceae of Illinois. This resulted in his first mycological publication, "Notes on North American Forms of Podosphaera," 1884. Although there is no exact record at present available, it is highly probable that young Earle assisted Professor Burrill in his epoch-making studies on the bacterial cause of pear blight, which were then being carried on, as his father's pear orchards must have furnished ample opportunity for observation and experiments with this disease.

Leaving the University in 1883, he returned home again to assist his father in his fruit-growing and shipping operations, utilizing his spare moments in collecting and studying fungi, especially the powdery mildews. In 1887, the result of this work, which was a monograph of the Erysiphaceae of the state, was published under the joint authorship of Burrill and Earle. He had an opportunity during this period to accept a good position at the Kentucky Agricultural Experiment Station but declined, believing that his duty to his father and their fruit interests, then being developed in southern Mississippi, demanded his first consideration.

In 1894 and 1895, he became connected with the Mississippi Agricultural Experiment Station, and in 1895–96 he acted as Assistant Pathologist in Charge of the Mycological Herbarium of the United States Department of Agriculture. This position, again, gave him an opportunity to devote his time entirely to fungi, but in 1896 his horticultural interests and perhaps financial considerations also led him to accept the position of Horticulturist at the Alabama Agricultural Experiment Station. A little later he was made Professor of Biology at the Alabama Polytechnic Institute from which, in 1902, he received the degree of M.S. The same year he accepted a call to the New York Botanical Garden to become Assistant Curator in Charge of Mycological Collections. Here he had an opportunity to do some of his best work in systematic mycology, giving special attention to the Agarics. This was interrupted, however, in 1904, by his appointment as Director of the Estacion Central Agronomica of Cuba. He brought to his assistance in this position a group of plant and animal investigators who inaugurated research of great importance to Cuban agriculture. Owing to political changes he resigned from his position in 1906 and purchased a farm at Herradura, Cuba, where he took up fruit and truck growing, which, in spite of his numerous other activities, he continued to carry on and enjoy until the end of his life.

From 1908 to 1911 he was Consulting Agriculturist of the Cuban-American Sugar Company and, during 1911–14, President of the Cuba Fruit Exchange. In 1918, he was sent by the United States Department of

Agriculture to Porto Rico to make an investigation of the mosaic disease of sugar cane, at that time threatening the destruction of the industry. In this connection his discovery of a variety of cane practically immune from the disease and his introduction of this variety into Porto Rico were of the greatest service to the sugar planters.

In 1922–23, he was Consulting Agriculturist to the Central Aguirre Sugar Company of Porto Rico. He next returned to Cuba and became Advisor for the General Sugar Company. In 1925, he accepted a position with the Tropical Plant Research Foundation and took charge of the study of sugar-cane varieties, which was continued until his death.

The results of his twenty-years observation and experience with sugar cane were embodied in a book entitled "Sugar Can and Its Culture," which is recognized as an authoritative work on the subject. Although engaged for the greater part of his life in commercial and economic phases of agriculture and horticulture, he always retained a very deep interest in mycological and pathological work and never lost an opportunity to increase his knowledge of the subject. He at one time prepared manuscript for a work on plant pathology which he had hoped to complete and publish, but, unfortunately, this, as well as his library, was destroyed in a fire which consumed his home in Herradura.

During his sugar-cane investigations in Cuba and Porto Rico he frequently found time to collect fungi, many of which were sent to the writer and have contributed to our knowledge of the occurrence and distribution of various species found on these islands.

He was a member of the American Association for the Advancement of Science, the Torrey Botanical Club, The American Phytopathological Society, the Botanical Society of America (President, 1906), and for many years Associate Editor of Mycologia. A genus of Rusts, Earlea, has been named in his honor and several species of fungi bear his name.

The character and extent of Professor Earle's scientific work and studies are indicated by the extensive bibliography here appended. A mere catalogue of this kind, however, throws little light upon his personal characteristics. He was of optimistic temperament and a genial companion. One of my pleasantest memories is of a visit to his home in Herradura and our discussions of various mycological, pathological, and miscellaneous subjects. His sterling character, quiet and retiring manner, and friendly disposition caused him to be greatly respected by his business associates and acquaintances and beloved by his more intimate colleagues and friends.

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PHYSIOLOGIC SPECIALIZATION AND MUTATION IN PHLYCTAENA LINICOLA SPEG.[1]

H. A. RODENHISER[2]

INTRODUCTION

The pasmo disease of flax, caused by *Phlyctaena linicola* Speg., is known to have been present in the Upper Mississippi Valley a number of years. In 1928 the disease was epidemic in many fields in Minnesota, and losses as high as 20 per cent were reported. In the flax-rust nurseries at University Farm, St. Paul, Minn., and at the Coon Creek Substation, a large number of wilt- and rust-resistant selections were killed by pasmo.

Cultures were obtained from diseased plants grown in different localities in Minnesota and the cultural characters studied. The cultural characteristics of some isolations were similar to those described by Brentzel (3) for *P. linicola* but others were entirely different. Furthermore, variant sectors appeared in some colonies of monosporous origin. These facts seemed to indicate the existence of physiologic forms of the pasmo pathogene and the origin of new forms by mutation.

A study, therefore, was made of different physiologic forms in order to determine their cultural characteristics, their stability, and possible origin, as well as their pathogenicity.

MATERIALS AND METHODS

During 1928 collections of diseased flax were made in several localities in Minnesota. Diseased portions of the flax stems in which pycnidia had developed were placed in a mercuric bichloride solution, 1:1000, for three minutes and then washed in sterile distilled water. Pieces were then transferred to potato-dextrose agar, and in about five days pure cultures of *P. linicola* were obtained. From these cultures single conidia were isolated by the method described by Hanna (10) and subcultures of the colonies that developed were used in all the tests. For identification of physiologic forms the cultures were first incubated for 10 days in test tubes on equal quantities of 2 per cent potato-dextrose agar. Small, approximately equal portions of each culture were then transferred for comparative tests to equal quantities

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[2] The writer wishes to express his appreciation to Dr. E. C. Stakman for help and criticism in preparing this paper.

of several different media in petri dishes or flasks of uniform size. The following characters were used in differentiating the forms on culture media: Color, topography, character of surface, consistency, and type of margin. The colors were classified according to Ridgway's "Color Standards and Color Nomenclature" (13).

Each physiologic form was designated by the locality whence it was obtained, together with an accession letter. The first form isolated in Minnesota, for example, was designated Minn. A, the second Minn. B, etc. Physiologic forms isolated from sectors are designated according to the colony in which they appeared, together, with an accession number. Thus, the first form obtained from a sector of Minn. A is Minn. A1; the second sector in Minn. A is designated as Minn. A2. Sectors occurring in Minn. A2 would be referred to as Minn. A2–1, Minn. A2–2, etc.

CULTURAL STUDIES

Several investigators (4, 5, 6, 7, 8, 12, 15) have found cultural and physicochemical differences between lines within species of various fungi. In colonies of some of these lines, sectors appeared which differed from their parent colonies in color, type of growth, and certain other characters. Christensen (6) found this to be true in monosporous cultures of *Helminthosporium sativum* P., K., and B., as did Hanna (11) in monosporous cultures of *Ustilago zeae* (Beck.) Unger.

Up to the present time eleven distinct physiologic forms of *P. linicola* have been obtained either from original isolations from diseased stems or as

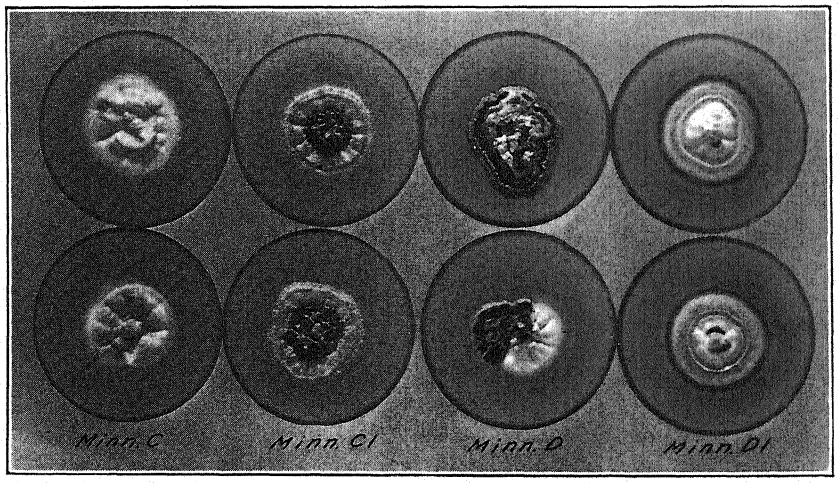


FIG. 1. Four physiologic forms of *Phlyctaena linicola* on potato-dextrose agar for 30 days. Note the fan-shape mutant developed in Minn. D.

TABLE 1.—*Cultural characteristics of physiologic forms of Phlyctaena linicola on potato-dextrose agar 30 days after inoculation*

Source of collection	Form	Color	Topography	Surface	Consistency	Margin	Conidial production
St. Paul, Minnesota	Minn. C	White to pale gull gray; center deep gull gray	Slightly umbilicate; few radial depressions	Velvety	Mycelioid	Slightly undulate	Slight
Sector from Minn. C	Minn. C1	White to pale olive-buff; center olive brown	Umbonate; center coral-like	Center waxy; ray powdery	Center leathery; ray mycelioid	Entire	Moderate
Crookston, Minnesota	Minn. D	Dusky green to glaucous-green interspersed with patches of gull gray	Raised to convex	Felty, interspersed with few very small wet blister-like areas	Mycelioid and bacterioid	Slightly lobate	Abundant
Sector from Minn. D	Minn. D1	Center white surrounded by band of neutral gray; remainder white to pale gull gray	Umbonate	Velvety	Mycelioid	Entire	Slight

mutants occurring in the form of sectors in these parental cultures. The data on cultural characteristics of four of these forms are summarized in table 1 and a photograph of the colonies is shown in figure 1. Marked differences in the color of the cultures were observed, not only between those of physiologic forms isolated directly from diseased flax but also between parent cultures and mutants. For example, under the conditions of the experiment, Minn. C was white to pale gull gray, with a center of deep gull gray. A mutant from this culture, Minn. C1, was white to pale olive-buff with a center of olive brown. The topography of the colonies varied from flat to umbilicate, the surface from dull to velvety and waxy, the consistency mycelioid to leathery, and the margin entire to slightly lobate. When grown under the same environmental conditions the cultural characteristics of any particular form were very constant (Fig. 1). On the other hand, the same forms grown at different temperatures and on different media were quite different in their cultural characteristics. Similar facts have been observed by Christensen (6) for *Helminthosporium sativum,* by Christensen and Stakman (8) for *Ustilago zeae,* and by Rodenhiser (14) for some of the cereal smuts. There is a difference also in the degree to which certain of the forms produce conidia. For example, Minn. D produced conidia abundantly, while Minn. C was almost entirely mycelioid and relatively few conidia were formed. Minn. D1, isolated from a sector in Minn. D, likewise was mycelioid and produced very few conidia. These cultures were transferred every three weeks, for one year, and this difference in conidial production remained constant.

INFLUENCE OF TEMPERATURE ON THE AMOUNT OF RADIAL GROWTH OF COLONIES

Tests were made to determine whether physiologic forms of *P. linicola* could be distinguished by differences in reaction to temperature. To each of a triplicate series of Erlenmeyer flasks of 250 cc. capacity were added 35 cc. of the same preparation of 2 per cent potato-dextrose agar. The flasks were inoculated with small, approximately equal portions of each culture and incubated at room temperature for 24 hours. All of the forms were then incubated at each of the following temperatures: 7°, 12°, 17°, 22°, 27°, and 32° C. The diameter of each colony was measured at the end of 30 days. The results of the experiment with six physiologic forms are summarized in table 2 and the data are graphically represented in figure 2. Brentzel (3) found the cardinal temperatures of *P. linicola* to be about 5°, 21°, and 32° C. From the data presented in table 2 it may be seen that the results obtained by the writer are corroborative. It seems clear, also, from these data, that physiologic forms of the organism react differently at certain temperatures. At the end of 30 days incubation at 17° C. the average diameter of colonies of Minn. C was 40.7 mm., while those of

TABLE 2.—*The influence of temperature on the growth of six physiologic forms of Phlyctaena linicola on potato-dextrose agar for 30 days*

Form	Temperature in degrees C. and diameter of colonies in mm.																			
	7				12				17				22				27				32			
	Series				Series				Series				Series				Series				Series			
	1	2	3	Av.	1	2	3	Av.	1	2	3	Av.	1	2	3	Av.	1	2	3	Av.	1	2	3	Av.
Minn. A	9	7	5	7.0	12	18	17	15.7	26	31	26	27.7	35	—	29	32.0	25	25	28	26.0	0	0	0	0
Minn. B	10	12	11	11.0	20	20	—	20.0	22	18	—	20.0	27	25	—	26.0	25	26	17	22.7	0	0	0	0
Minn. C	10	11	9	10.0	20	22	19	20.3	40	40	42	40.7	44	44	45	44.3	22	26	26	24.7	tr	tr	tr	tr
Minn. C1	10	10	15	11.7	24	19	21	21.3	28	29	—	28.5	47	45	48	46.7	21	24	23	22.7	0	0	0	0
Minn. D	11	10	9	10.0	20	15	15	16.7	18	20	23	20.3	32	25	32	29.7	18	23	21	20.7	tr	tr	tr	tr
Minn. D1	11	10	10	10.3	18	16	17	17.0	24	25	26	25.0	47	44	45	45.3	30	31	30	30.3	tr+	tr+	tr+	tr+

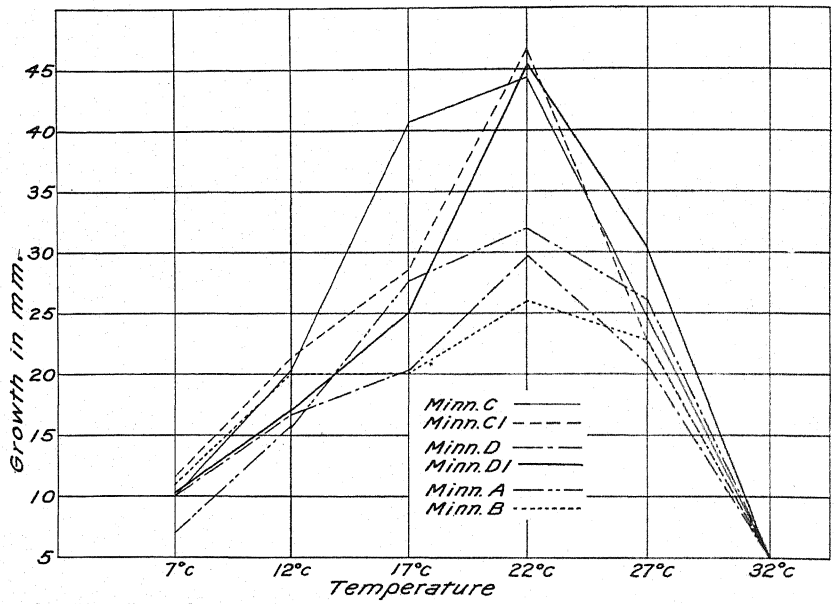


FIG. 2. The effect of temperature on the growth of six physiologic forms of *Phlyctaena linicola* on potato-dextrose agar for 30 days.

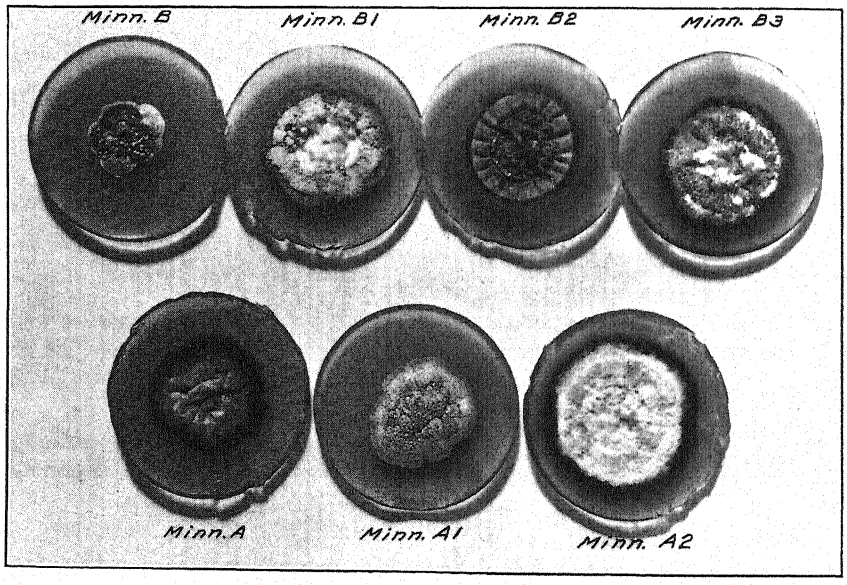


FIG. 3. Cultures of physiologic forms of *Phlyctaena linicola* showing differences in characteristics between parents and mutants. Minn. B and three mutants. Minn. A and two mutants.

Minn. B and Minn. D were 20.0 mm. and 20.3 mm., respectively. When incubated at certain temperatures there were differences in the amount of radial growth of parent colonies and mutants. When incubated at 17° C. the diameters of colonies of Minn. C averaged 40.7 mm., while those of Minn. C1 averaged 28.5 mm. At 22° C. there was a difference of only 2.4 mm. in their average diameter, the colonies of Minn. C1 being slightly larger than those of the parent, Minn. C. In general, cultures of mutants grew more rapidly than parental cultures, but not always. At 12° C. colonies of Minn. D and the mutant Minn. D1 reacted approximately the same, but at 17°, 20°, and 27° C. the average diameters of the parental colonies were much smaller than those of the mutant, the differences being 4.7 mm., 15.6 mm., and 9.6 mm., respectively.

VARIABILITY AND MUTATION

The range of variability in cultural characters of physiologic forms is very large (Figs. 1, 3). By changing the environment under which the cultures are grown decided temporary modifications can be induced. The genotypic constitution does not seem to be changed, however, as the modifications assume the original characters when again grown under fairly normal conditions. Furthermore, under the same environmental conditions the characters of any given line are remarkably constant. Similar facts have been observed for *Helminthosporium sativum* (7), for *Ustilago zeae* (8), for some of the cereal smuts (14), and for *Sphacelotheca sorghi* (9). There seems to be every reason to suppose from the observations made by the writer that the physiologic forms of *P. linicola* differ from each other genotypically and that these characters, although subject to considerable fluctuation, are constant under uniform conditions. When new physiologic forms appear they seem to arise in the form of definite sectors in colonies (see Fig. 4).

The sectors in colonies of *P. linicola* were usually wedge- or fan-shape, although in two instances new forms were isolated from irregularly outlined patches that appeared near the center of the parental colony. When transfers were made from these variants, repeated sectoring took place in the resulting colonies to the extent that there were sectors within sectors. The cultures obtained from these sectors retained their characteristics for a considerable period of time and when there were reversions to the parental type they came about as a result of sectoring.

The question arises as to whether the sectors described represent mutations. Several investigators (2, 5, 6, 7, 8, 15) have described mutations in fungus colonies growing on culture media. It is beyond the province of this paper to discuss in detail the arguments for and against the designation of sectors in fungus colonies as mutants. If one accepts Baur's (1, p.

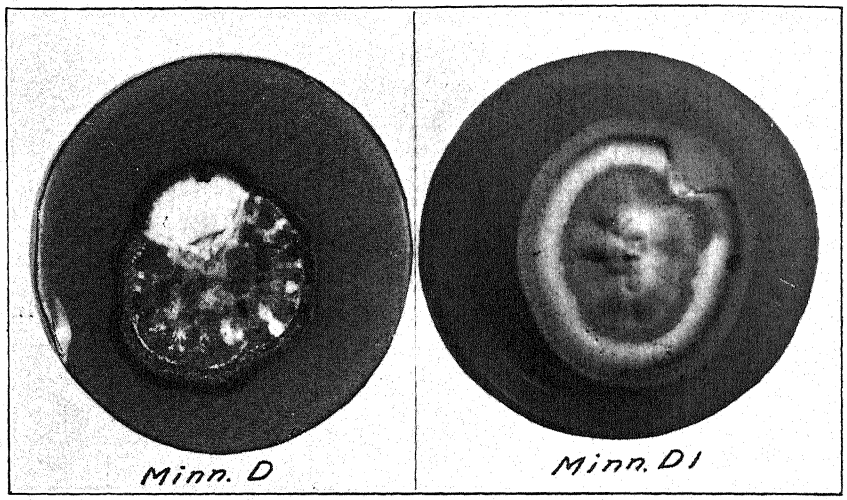


FIG. 4. Mutants arising as distinct sectors in colonies of *Phlyctaena linicola*. Minn D1 was isolated from the sector in Minn D. Note that Minn D1 is again sectoring.

436) definition of mutation, that is, heritable differences between parents and their offspring, including those that have originated asexually, which do not arise through hybridization but which have other causes, it would seem justifiable to designate as mutants the new lines of *P. linicola* which have been isolated as sectors in colonies of monosporous lines. The differences in cultural characteristics between the parents and the so-called mutants certainly have persisted through a number of asexually propagated generations and no anastomosing of hyphae or fusions between conidia ever have been observed. It seems unlikely, therefore, that the sectors are the result of chance assortment of nuclei. This seems particularly true because the individual cells of the conidia are uninucleate. Whether one designates as mutation or saltation the process by which new physiologic forms originate in the form of sectors is probably unimportant. It does seem significant, however, that new physiologic forms actually are originating in this manner.

PATHOGENICITY

An attempt was made to find out whether these forms differed in pathogenicity also. In the greenhouse the following flax varieties and selections were inoculated with four culturally distinct forms: Winona C. I. 179, Chippewa C. I. 178, Ottawa C. I. 24, Argentine (C. I. not known), and an Argentine-by-Saginaw cross. Water suspensions of cultures of Minn. C, Minn. C1, Minn. D, and Minn. D1 were used and approximately equal amounts of the inoculum sprayed on the flax plants with a Daisy hand

sprayer. Four pots of each variety or selection, containing six plants each, were inoculated with the individual physiologic forms, respectively, about the time of boll formation. After an incubation period of nine days the characteristic lesions appeared on the leaves and two weeks later they were well developed on the stems. In no case did there appear to be an appreciable difference in the pathogenicity of the different physiologic forms. Several leaves on all of the plants were infected and from three to five lesions developed on each stem. The symptoms on the Argentine plants developed two days before those on the other varieties or selections.

Nine varieties and selections of flax were inoculated in the field with two physiologic forms of *P. linicola,* as indicated in table 3. Approxi-

TABLE 3.—*Summary of data on tests for pathogenicity of two physiologic forms of Phlyctaena linicola at University Farm, St. Paul, Minn., in 1929*

Variety or selection of flax	C. I. No.	Degree of infection from					
		Minn. D		Minn. D1		Noninoculated	
		Series		Series		Series	
		A	B	A	B	A	B
Red Wing	480	H[a]	H	L–	L–	T	O
Winona	179	H–	H	T	T+	O	T
Slope	274	M	M+	L+	L	O	T
Buda	336	M	M	T	T+	O	O
Bison	389	M	M	L–	L–	T	T
Linota	433	M+	M	T	T+	T	T
N. D. R. 114	268	M+	M+	T	T+	O	T
Reo (L. 79)	280	M+	H	L	L–	L	T+
Argentine (Selection)	—	H	H	T+	L–	T	T+

[a] H = heavy; M = moderate; L = light; T = trace.

mately 125 seeds of each variety or selection were planted in duplicate seven foot rows, replicated twice. After the bolls had formed, the plants in one series were sprayed five times at intervals of four days with water suspensions of cultures of Minn. D and in a duplicate series with Minn. D1. From the data presented in table 3 it would seem that Minn. D is more virulent than Minn. D1. In the greenhouse tests, however, no difference was found in the pathogenicity of these two physiologic forms. An explanation of the differences in degrees of infection obtained in greenhouse and field trials may be as follows: Minn. D in culture produces conidia abundantly, while Minn. D1 produces few conidia and is almost entirely mycelioid. In the greenhouse, a short time after the lesions appeared on the leaves and stems the plants dried up and, consequently, lesions resulting

from secondary infections did not develop. In the field, conditions for secondary infection were optimum and plants inoculated with a physiologic form that produces conidia abundantly would be apt to be more heavily infected than those plants inoculated with a form such as Minn. D1 which produces relatively few conidia. From the data presented in table 3 it will be noted also that certain varieties and selections in the check series became infected. Unfortunately, the seed for this series was sown near some very susceptible Argentine selections wherein pasmo developed as a result of natural inoculation. When preliminary notes were taken the disease had not spread to the check series but, when the final readings were made, lesions had developed on the leaves. It is presumed that the inoculum came from the adjacent plots of Argentine flax.

VARIETAL RESISTANCE

In 1928, in the flax-rust nurseries at University Farm, St. Paul, Minn., and at the Coon Creek substation, and, again, in 1929, at University Farm, many selections of flax, highly resistant to wilt and rust, were killed by pasmo. In the nurseries several commercial flax varieties were grown and, in addition, about six hundred selections of the following crosses: Argentine by Saginaw, Argentine by Winona, Saginaw by Ottawa, Winona by Ottawa, Winona by Bombay, Winona by Montevideo, Blue Blossom Dutch by Bombay, and Saginaw by Bombay. In the commercial varieties the degrees of resistance varied; the Argentine flaxes were completely susceptible, while Red Wing C. I. 480, Winona C. I. 179, Chippewa C. I. 178, Linota C. I. 433, and North Dakota C. I. 268 were moderately so. Buda C. I. 336 and Bison C. I. 389 were resistant. In the selections from the various crosses there were all gradations from a high degree of resistance to complete susceptibility. Resistance, then, is not correlated with any particular agronomic type, and it should be possible to breed varieties of flax possessing desirable agronomic characteristics and high resistance to pasmo.

DISCUSSION AND CONCLUSIONS

There are at least several physiologic forms of *P. linicola* that differ culturally, in temperature relations, in conidial production, and to a certain extent in pathogenicity. Not only are there several forms now extant but, no doubt, new ones are continually being produced; at least, they arise on culture media by the process of mutation, if the sectoring in colonies can properly be designated as mutation. That these so-called mutants are not mere temporary variants is indicated by the fact that their characters remain remarkably constant under uniform conditions except as changes again may occur as a result of sectoring. It seems quite likely that the differential effect of temperature on different physiologic forms may deter-

mine, to a considerable extent, which forms are likely to predominate in certain regions or in certain seasons. The further fact that some forms produce conidia far more abundantly than others probably enables the former to do greater damage because of their ability to cause secondary infections. There apparently are all gradations in susceptibility among varieties of flax ranging from complete susceptibility to high degree of resistance. Resistance does not seem correlated with any particular agronomic type, and it should therefore be possible to breed varieties that combine desirable agronomic characters with resistance to pasmo.

SUMMARY

1. *Phlyctaena linicola* comprises distinct physiologic forms that can be distinguished in culture by the following characters: Color, topography, surface, consistency, type of margin, and the degree to which conidia are produced.

2. The differences between physiologic forms in culture sometimes are very great. When grown under the same environmental conditions, the cultural characteristics are constant. Temporary modifications may be induced but the genotypic constitution is not changed, for the variants assume their original characteristics when the forms are again grown under normal conditions.

3. The temperature relations of different physiologic forms differ. This is indicated by the fact that the amount of radial growth differs greatly at different temperatures.

4. Wedge-shape sectors appear commonly in colonies of the organism on culture media. The cultures isolated from these sectors retain their distinctive characters for long periods of time and the writer considers the preponderance of evidence to indicate that they are true mutants.

5. The mutants are as stable as the forms originally isolated from the diseased plants.

6. Physiologic forms of the pasmo organism differ in the degree to which they produce conidia. Under experimental conditions, secondary infections were most numerous when forms were present that produced conidia abundantly. The number of secondary infections was far lower when the form present produced relatively few conidia.

7. Flax varieties and selections varied considerably in their resistance to pasmo. All gradations of resistance and susceptibility were found. None of the lines were found to be immune.

8. There seems to be no correlation between agronomic type in flax and resistance to pasmo. It should be possible, therefore, to breed resistant varieties of the desired agronomic type.

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PURIFICATION AND CERTAIN PROPERTIES OF THE VIRUS OF TYPICAL TOMATO MOSAIC[1]

P. H. Brewer, H. R. Kraybill, R. W. Samson, and M. W. Gardner

Various methods (4) have been employed in attempting to free the tomato-mosaic virus as completely as possible from the different constituents of the plant juice and the best success has been obtained by supercentrifuging the juice expressed from large quantities of minced mosaic-plant tissue, discarding the liquid, which contains most of the soluble constituents of the cell sap, and releasing the virus from the solid residue in distilled water, supercentrifuging a second time, and clearing and decolorizing the liquid thus obtained by the use of aluminum-gel acid in reaction (5).

Clear and colorless virus suspensions thus prepared have been found to lose their virulence after passage through atmometer cylinders, Pasteur-Chamberland B filters, and collodion filters and after heating to 84° to 88° C., and, in general, to remain active for long periods under conditions of refrigerator storage. The virus is inactivated when the suspension is made more alkaline than pH 8.5 and reactivated when the acidity is restored. Evidence was obtained that the virus, under the influence of the electric current, passed to the positive pole.

MOSAIC VIRUS OBTAINED FROM RESIDUE IN SUPERCENTRIFUGE AND PURIFIED WITH ALUMINUM GEL

To obtain the mosaic virus a number of tomato plants affected with the typical mosaic [Type A of Fernow (7), Tobacco Virus 1 of Johnson (9)] are ground in a food chopper and the juice expressed through heavy muslin in a Cossette porcelain press. This juice is diluted with an equal volume of water and passed through a Sharples laboratory supercentrifuge three times at the rate of 12, 6, and 3 liters per hour, respectively, and at about 35,000 revolutions per minute. The liquid, which contains most of the soluble constituents of the plant juice, and which, according to McKinney's supercentrifuge tests with tobacco (13, p. 34), should contain relatively little of the virus, was discarded. The dark green, gummy residue, which in McKinney's tests (13, p. 34) contained most of the virus, is scraped from the bowl of the centrifuge, resuspended in distilled water by

[1] Contribution from the State Chemist and Botany Departments, Purdue University Agricultural Experiment Station, and the Agronomy Department, School of Agriculture, La Fayette, Indiana.

stirring with a Bouyoucos deflocculator, and again passed through the supercentrifuge. The residue is discarded. To the centrifuged liquid, which is somewhat yellowish and cloudy, is added aluminum gel prepared so as to have a slightly acid reaction, a precaution which prevents it from inactivating the virus, as in Allard's tests (2). By adsorption the aluminum gel removes the suspended solids and the yellowish color and, when the mixture is filtered, the filtrate is a clear and colorless suspension of the virus which upon inoculation proves as infectious, apparently, as the juice originally expressed from the plants.

In the course of this work many virus suspensions have been thus prepared and purified and nearly all have given 100 per cent mosaic infection when inoculated into sets of 10 or 15 young tomato plants grown in an insect-free greenhouse.

Inoculation is performed by means of small glass tubes drawn out to a narrow point. Rather large numbers of these small pipettes, washed and sterilized, are kept on hand and a fresh pipette is used for each plant inoculated. By inserting the point of the pipette into the virus suspension in a test tube, a small amount is drawn up into the pipette by capillarity. In performing the inoculation, two or three leaves of each plant are scratched about three times with the point of the pipette from which a small amount of the virus suspension escapes into the wound as it is made. While the leaf is being inoculated it is supported on a small piece of paper toweling to prevent contact with the hand. By using a fresh pipette and paper for each plant there is no danger of accidental transfer of a virus from one plant to another. In the course of this work there has been a minimum of accidental infection. Series of control plants not handled at all, or inoculated with tap water, have remained free from infection.

With clear and colorless virus suspensions freed from all of the coarser solids and most of the soluble constituents of the plant juice and yet remaining actively infectious, it is believed that the various properties of the virus may be more accurately determined.

MOSAIC VIRUS FILTERED OUT BY ATMOMETER CYLINDER, PASTEUR-CHAMBERLAND B, AND COLLODION FILTERS

Virus suspensions purified by the method just outlined were found to be infectious after passage through a Pasteur-Chamberland F filter and Schleicher and Shüll 1½ per cent collodion filters, but not after passage through a Pasteur-Chamberland B filter, a cylindrical porous cup atmometer, Schleicher and Shüll 3, 4½, 6, and 7½ per cent collodion filters, and at 20 inches pressure and pH 4.80 through 2, 3, and 5 per cent collodion filters made with equal amounts of alcohol and ether as a solvent by pouring the

solution onto a mercury surface and exposing to the air 15 to 20 minutes before adding water. The results of the filtration tests on preparations of proved infectiousness are summarized in table 1.

After a filtration was completed the top of each of the 2, 3, and 5 per cent collodion filters was washed with distilled water and with this wash water from each filter a set of ten plants was inoculated and all of the plants in each set developed mosaic. This showed that the virus, in part at least, was retained in an easily removable condition on the filter.

TABLE 1.—*Filtration tests with purified virus of tomato mosaic*

Filter used	Plants inoculated	
	Total number	Number mosaic
Pasteur-Chamberland B	30	0
" " F	85	64
Atmometer cylinder	20	0
Schleicher and Shüll 1½%	40	9
" " 3 %	30	0
" " 4½%	40	0
" " 6 %	30	0
" " 7½%	30	0
Collodion 2 %	10	0
" 3 %	40	0
" 5 %	20	0

The failure of the virus to pass through collodion filters is in accordance with the results obtained by Kraybill and Eckerson (11) with Schleicher and Shüll and laboratory-prepared filters and, for the virus as it occurs in tobacco, by Duggar and Karrer (6), and by Mulvania (14) with filters made up with a similar proportion of alcohol in the solvent. Klebahn (10, p. 84), however, found that the virus from tobacco passed through a 2 per cent collodion filter. In his very early investigation of this virus as it occurs in tobacco, Iwanowski (8) found that it passed through a Chamberland filter. The failure of the virus to pass through the atmometer is in accordance with the results obtained by Allard (2) and Duggar and Karrer (6) with the same virus in tobacco.

The content of solids in two active filtrates from Pasteur-Chamberland F filters was found to be 0.00147 gram and 0.0018 gram per cubic centimeter or about 0.15 per cent. Examination of the original virus suspension with an ultramicroscope showed numerous particles which became progressively less abundant in the filtrates from the collodion filters as the percentage of collodion was increased. No particles whatever were visible in the filtrates from the filters containing the two highest percentages of collodion (6 per cent and 7½ per cent).

MOSAIC VIRUS ACTIVE AFTER LONG PERIODS OF REFRIGERATOR STORAGE

The virus of typical tomato mosaic in extracted plant juice is known to be exceptionally long-lived (1, 9). Storage tests at ordinary refrigerator temperatures (36°–40° F.), the results of which are presented in table 2, have shown that, in general, the purified virus suspensions were still active after 6 to 20 months. No preservative was added. Four of the nine preparations tested (Nos. 2, 4, 5, and 6) apparently weakened with age, one (No. 6) apparently having lost its virulence in 564 days.

TABLE 2.—*Effect of refrigerator storage on virulence of purified tomato-mosaic-virus suspensions*

Virus suspension	Age	Plants inoculated	
		Total number	Number mosaic
1	Fresh	15	15
	110 days	15	10
	249 "	10	9
2	Fresh	15	15
	96 days	15	8
	234 "	10	5
3	Fresh	15	15
	86 days	15	8
	225 "	10	9
	606 "	10	10
4	Fresh	15	15
	74 days	15	10
	213 "	10	6
5	Fresh	15	15
	47 days	15	7
	187 "	10	7
	567 "	10	3
6	Fresh	15	15
	44 days	15	8
	184 "	10	8
	564 "	10	0
7	Fresh	15	15
	613 days	10	10
8	Fresh	10	9
	275 days	10	10
9	Fresh	10	10
	273 days	10	10

MOSAIC VIRUS INACTIVATED AT ABOUT 88° C.

Ten-minute exposures of the purified virus suspension in test tubes to temperatures of 70° to 90° C. indicated that the virus was inactivated at

about 88° C., as has been previously found by Allard (2), Johnson (9), and McKinney (12). For example, in one series of tests in which 15 plants were inoculated from each test tube of the heated virus suspension, the tube heated at 80° C. yielded 14 mosaic plants, at 82°, 12 mosaic plants, at 84°, 6 mosaic plants, at 86°, 2 mosaic plants, at 88°, no infection, and at 90°, no infection.

Longer exposures at the lower temperatures tended to destroy the virus. For example, out of 30 plants inoculated with virus suspensions heated 25 minutes at 70° C., 23 developed mosaic; out of 40 plants inoculated from suspensions heated 25 minutes at 74° C., 28 developed mosaic; out of 60 plants inoculated from suspensions heated 25 minutes at 78° C., only 18 developed mosaic; out of 60 plants inoculated from suspensions heated 25 minutes at 82° C., only 6 developed mosaic; and none of the 20 plants inoculated from suspensions heated 25 minutes at 84° C. developed mosaic.

TABLE 3.—*Inactivation of mosaic virus by increasing alkalinity*

H-ion concentration		Plants inoculated	
Original pH	Final pH	Total number	Number mosaic
5.25	5.25	10	9
	6.01	10	8
	7.03	10	7
	7.53	10	6
	8.12	10	6
	8.50	10	6
	8.75	10	0
5.33	5.33	10	10
	6.69	10	6
	7.61	10	4
	8.04	10	0
	8.40	10	0
6.43	6.43	15	15
	7.14	15	15
	7.44	15	15
	7.53	15	14
	7.77	15	0
	7.87	15	0
	8.12	15	0
7.11	7.11	15	15
	7.28	15	14
	7.56	15	15
	7.73	15	4
	8.04	15	2
	8.29	15	0

MOSAIC VIRUS INACTIVATED WHEN SUSPENSION MADE ALKALINE AND REACTIVATED WHEN ACIDITY RESTORED

The hydrogen-ion concentration of the purified virus suspensions as determined with the quinhydrone electrode usually fell within the limits of pH 5.0 to pH 6.5. When the hydrogen-ion concentration of the virus suspension was changed by adding dilute HCl or NaOH it was found that the virus was inactivated when the suspensions were made more alkaline than pH 7.5 to pH 8.5, as shown in table 4, and was activated again when the acidity was restored (Table 5). A slight precipitate was produced at about the same point at which inactivation occurred. The virus was not inactivated by an increase in acidity up to pH 2.46. Allard (3) found that the virus as it occurs in tobacco was very resistant to acids and sensitive to NaOH.

The inactivation of the virus by increasing the alkalinity of the preparation might be explained by assuming that the virus or virus-bearing particles are negatively charged, an explanation which is substantiated by cataphoresis tests to be mentioned later.

TABLE 4.—*Reactivation of mosaic virus by restoring acidity*

H-ion concentration		Plants inoculated	
Original pH	Final pH	Total number	Number mosaic
8.50	8.50	15	0
	8.12	15	6
	7.37	15	13
	5.66	15	14
8.40	8.20	10	0
	8.13	10	0
	8.04	10	0
	7.74	10	1
	5.33	10	6

IN ELECTRIC FIELD MOSAIC VIRUS TENDS TO ACCUMULATE AT POSITIVE POLE

With purified preparations to which dilute KCl was added preliminary cataphoresis tests were carried out in a U tube with a direct current of 110 volts. After the current had passed through the preparation about 6 hours, inoculations were made with specimens of the liquid drawn off from either pole. With one preparation, five tests may be summarized as follows: Only two of the 50 plants inoculated with the liquid from the negative pole developed mosaic, while 37 of the 50 inoculated with the liquid from the

positive pole developed the disease. These results indicate that the virus is carried toward the positive pole, as Olitsky and Hoffman (15) have recently shown, and that the virus or virus-bearing particles are negatively charged.

SUMMARY

Clear and colorless suspensions of the tomato-mosaic virus practically free from the various constituents of the plant juice have been prepared by discarding the liquid from the supercentrifuge and suspending the gummy residue in distilled water, centrifuging again to remove this residue, and clearing and decolorizing the resultant liquid with aluminum-gel acid in reaction.

Virus suspensions thus prepared were still active after passage through Pasteur-Chamberland F filters and 1½ per cent Schleicher and Shüll collodion filters but lost their virulence after passage through an atmometer cylinder, a Pasteur-Chamberland B filter, Schleicher and Shüll 3, 4½, 6, and 7½ per cent collodion filters, and 2, 3, and 5 per cent collodion filters precipitated from solution in equal parts of alcohol and ether. The wash water from the upper surface of used collodion filters was infectious.

The active filtrate from the Pasteur-Chamberland F filters contained about 0.15 per cent of solids.

Virus suspensions stored in a refrigerator, without a preservative added, in general proved to be infectious after 6 to 20 months.

Virus suspensions were inactivated by short exposures to 88° C. and by longer exposures to 82° and 84° C.

The hydrogen-ion concentration of the virus suspensions as prepared was usually about pH 5.0 to pH 6.5. The virus was not inactivated when the acidity was increased to pH 2.46.

When the alkalinity of the virus suspension was increased to about pH 7.5 to pH 8.5, a precipitate was formed and virulence was lost. Virulence was regained, however, when the suspension was made more acid.

When subjected to a cataphoresis test, the virus showed a tendency to be carried to the positive pole. Apparently, the virus or virus-bearing particles are negatively charged.

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HEART ROT OF PINEAPPLE PLANTS[1]

C. P. SIDERIS[2] AND G. E. PAXTON

INTRODUCTION

Young pineapple plants are susceptible to a destructive disease known as heart rot or stem rot. Diseased plants die with the partial or complete destruction of the meristematic tissues of the stem. Losses from this disease in Hawaii vary in different years and show some apparent correlation with abundance of rainfall. The distribution of the disease in the different fields is highly sporadic, certain fields losing from 10 to 30 per cent of their plants and others losing none. Pineapple heart rots have been reported from a number of widely separated countries.

In the Hawaiian Islands, the disease has been attributed by Kunkel (7) to a Phycomycete and by Lyon[3] to Phytophthora. In Jamaica, Ashby (1) obtained a Phytophthora from diseased pineapples, and in Australia a species of Phytophthora also was obtained by J. H. Simmonds (13).

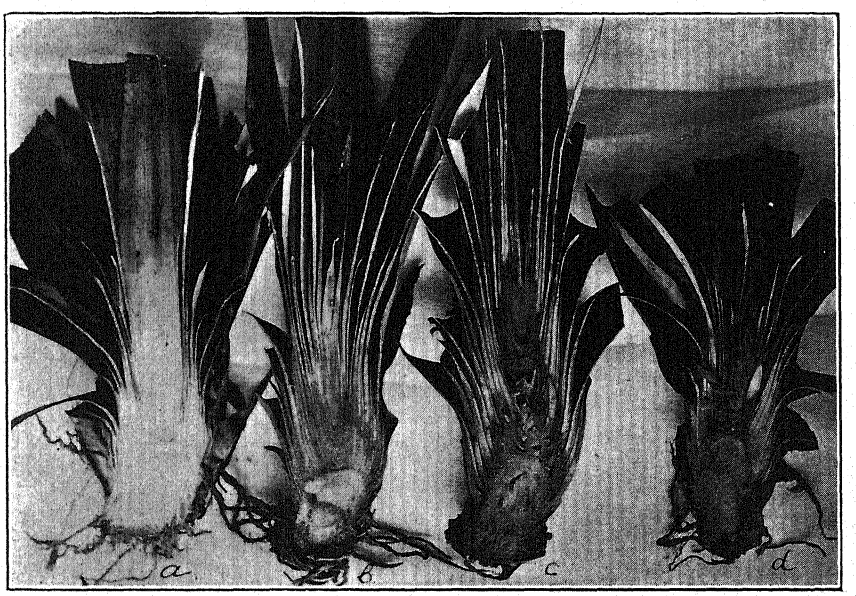


FIG. 1. Healthy and diseased pineapple plants. *a* healthy; *b*, *c*, and *d* diseased, infected with *Phytophthora Meadii*.

[1] Technical Paper No. 12 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

[2] Physiologist and formerly Pathologist of the Experiment Station of the Association of Hawaiian Pineapple Canners.

[3] Dr. H. L. Lyon's oral statement to the senior writer.

GENERAL SYMPTOMS OF THE DISEASE

The disease is characterized in the early stages of infection by loss of turgidity and a slight twisting of the central leaves and, in later stages, by the withering and discoloration to yellowish pink and brown. In both early and advanced stages the inner whorls of leaves can be readily detached from the stem by a slight pull, the basal tissues of all such leaves having undergone either a partial or complete disintegration (Fig. 1). Natural falling of these leaves is a frequent late symptom. The stem may also show either a partial or complete disintegration with a characteristic brownish yellow discoloration at the margins between healthy and diseased tissues (Fig. 2).



FIG. 2. Cross section of pineapple stem infected with *Pseudopythium phytophthoron.*

The initial infection may appear (1) at the apical end of the stem, (2) at the base of some leaf, (3) at the basal end of the stem, or (4) at the axillary or main roots. The infected tissues soon undergo decomposition. Saprophytic fungi and bacteria may invade such tissues after the initial invasion of the pathogene and cause putrefaction.

THE CAUSAL ORGANISMS

Three organisms were isolated from the diseased tissues of pineapple plants in Hawaii and, on inoculation, reproduced the typical symptoms of the disease. These were *Phytophthora Meadii* MacRae (8), *Ph. melongenae* Sawada (14), and *Pseudopythium phytophthoron* Sideris (12). The culture of *Ph. Meadii* obtained from pineapples is not a typical representative of the species, as comparative studies by the senior writer with an authentic culture[4] have shown. It constitutes a distinct strain distinguishable by a more vigorous development of aerial mycelium. The culture *Ph. melongenae* is a true representative of the authentic or type culture.

These three organisms have been isolated repeatedly from diseased pineapple plants. From the soil they have been isolated only indirectly. Susceptible planting material, tops or crowns, if set deeply enough to cover some of the leaves in soil from infested areas and kept watered to excess, usually will develop the disease. From such plants the fungi are readily isolated in pure culture.

Artificial inoculations with all three organisms were made by placing the inocula at the base of the leaves, on the stem or on the roots of the host grown in root-study boxes (5, 11). Such inoculations were repeated many times and reisolations of the organisms made from resulting diseased tissues. There is no reason to doubt the pathogenicity of these three fungi and their responsibility for the development of heart rot of pineapples.

COMPARATIVE BEHAVIOR OF THE THREE PATHOGENES

The macroscopic symptomatology of heart rot varies but slightly with the three organisms causing it. There are certain differences, of frequent but not constant occurrence, that distinguish the symptoms of heart rot caused by *Pseudopythium phytophthoron* from those caused by *Phytophthora Meadii* and *Ph. melongenae*. These lie in the fact that the former organism makes its initial infection most frequently through the roots and the latter two through the leaf bases. Stem and leaf rot, in the case of *Ps. phytophthoron,* develops after the leaves of the plants have become discolored and flaccid as a result of the destruction of the roots, whereas with *Ph. Meadii* and *Ph. melongenae* both discoloration of the leaves and rotting of

[4] Authentic cultures obtained from Centraal Bureau voor Schimmelcultures, Baarn, Holland.

the stem and leaves develop simultaneously. Consequently, it is possible to recognize the work of *Ps. phytophthoron* under field conditions by the severity of root rot which precedes actual heart rot.

Pseudopythium phytophthoron.—The pathological behavior of this organism is characterized by the wholesale killing of plants through the primary destruction of their root systems. The aerial parts of the plant also become invaded either indirectly, through the initial root infections or, directly, by invasion of the stem through the tender tissues of leaf bases. In cases where the parasite has invaded only the tissues of the roots and not those of the stem, the plants do not develop the characteristic symptoms of heart rot; that is, the leaves do not rot but become discolored and slightly twisted and withered. Typical symptoms of heart rot develop only in cases where the tissues of the stem and leaves have been invaded by the pathogene.

Pseudopythium, apparently, is very limited in its distribution in the Islands. It has been found in only one field, that being in the Waialua district on the Island of Oahu. Its present distribution is limited to an area of apparently 20 acres in that field. Some care has been exercised by the men in charge of this field to keep down the dissemination of the parasite as much as possible. This area, from which the organism was first obtained by the senior writer in 1925, has not grown a successful crop of pineapples since.

Observations in the field have indicated that temperatures below 70° F., together with abundant moisture, favor the development of this disease.

Phytophthora Meadii.—This organism was first obtained from the diseased tissues of pineapple plants by the senior writer in 1925. Since then

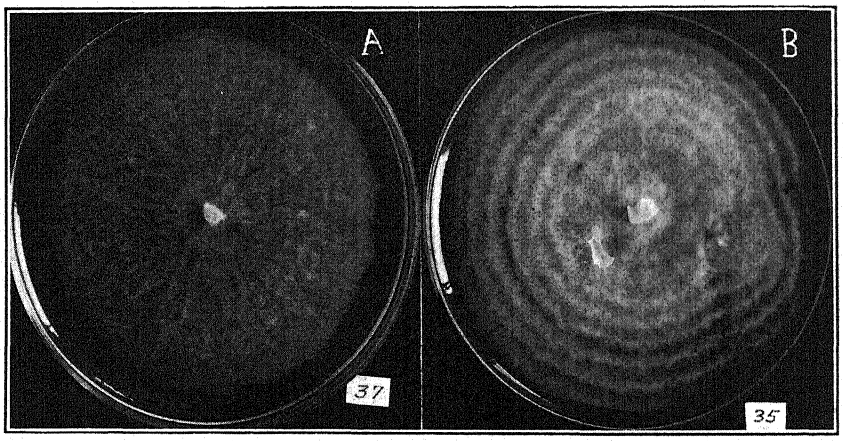


FIG. 3. A. Colony of *Phytophthora Meadii* (pineapple strain) on Carica papaya agar. B. Colony of *Ph. melongenae* on Carica papaya agar.

it has been isolated repeatedly from many localities on the islands of Oahu, Maui, and Molokai of the Hawaiian Archipelago. Of the three organisms studied, this is the one most frequently responsible for the heart rot in these Islands.

This organism produces heart rot chiefly in young plants one week to six months old. Initial invasion may take place through the axils of either basal or apical leaves, through the basal tissues of the stem, or through the

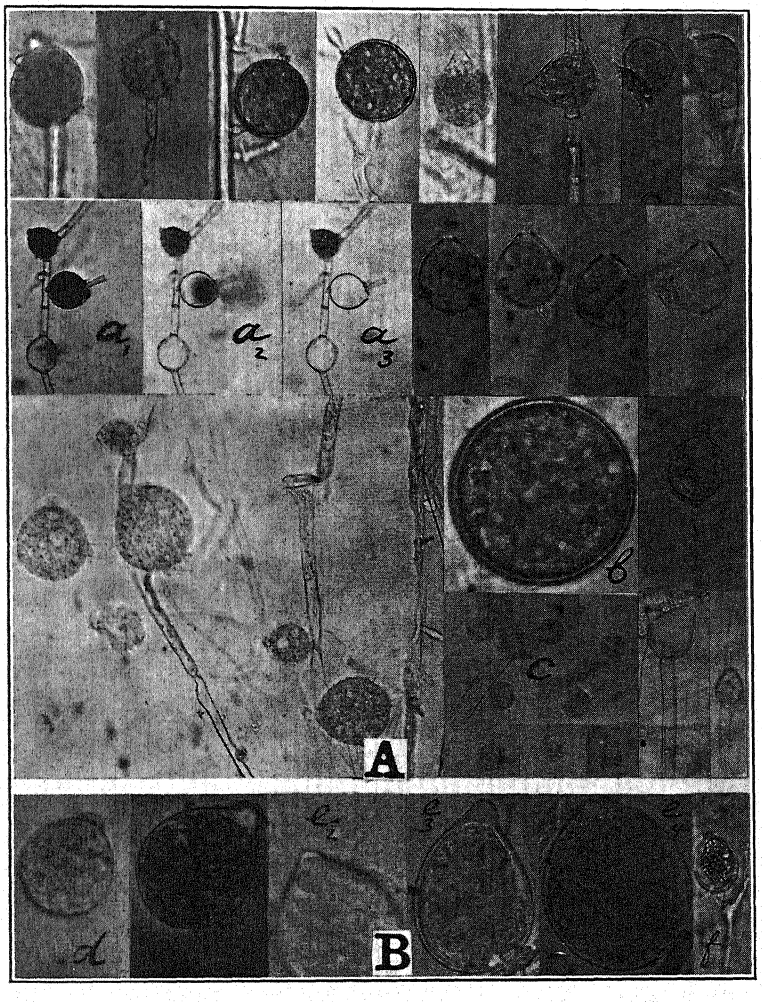


FIG. 4. A. *Phytophthora melongenae*: *a*, germination of zoosporangia (×150); *b*, pseudoconidium or resting spore (×600); *c*, zoospores (×300). Unlabeled illustrations are zoosporangia and euconidia and pseudoconidia (×300). B. *Ph. Meadii*: *d* and *e*, conidia (×600); *f*, conidium (×300).

roots, though the latter path of entry is much less conspicuous than with Pseudopythium. The pathogene lives in the soil and its transfer to leaf axils takes place through the agencies of winds, cultivation implements, and the splashing and overflow of water during heavy rains. In parts of fields where temporary flooding has occurred during heavy rains the disease is more abundant than elsewhere. The fungus, washed with wet soil into the axils of the leaves, is in a favorable position to grow rapidly and invade the tender tissues. The greatest development of the disease occurs during the winter months, which are both cooler and wetter than the summer. The influence of temperature and moisture has not been studied under controlled conditions, but the continued development of the disease in wet fields during the summer months suggests that moisture rather than temperature is the limiting factor.

Phytophthora melongenae.—This organism was first obtained in 1929 by the junior writer from the diseased tissues of pineapple plants grown on the Island of Lanai of the Hawaiian Archipelago. Previous to its isolation from pineapple tissues the senior writer had isolated it from the diseased tissues of *Antirrhinum majus* L. growing in Honolulu, and, on inoculation, it proved pathogenic on roots of *Ananas sativus* Schult.

Extensive studies have shown that this organism is even more aggressively parasitic than *Ph. Meadii.* The pathogenic behavior of these organisms is otherwise very similar, and the resulting symptoms are almost indistinguishable.

OTHER HOSTS OF THE HEART-ROT PATHOGENES

Pseudopythium phytophthoron, being a new organism, has not been studied extensively. *Allium cepa* L. is the only other plant thus far found susceptible.

Phytophthora Meadii was first obtained by MacRae (8) from the diseased tissues of the fruits of *Hevea braziliensis* Muell., causing fruit rot. According to MacRae, the organism is exceedingly destructive during very high humidity. Ashby (2) considers *Ph. arecae* (Colem.) Pethyb. a strain of *Ph. Meadii* and not a distinct species, both organisms occurring in the same geographical area. *Ph. arecae* has been obtained by Coleman from the diseased tissues of the leaves of *Areca catechu* L. or betel nut palm.

Phytophthora melongenae (14) was first obtained by its discoverer from the diseased tissues of the fruit of eggplant, *Solanum melongenae* L. Sawada found that not only the Formosan white eggplant but all other Japanese varieties are susceptible to this disease. Inoculation tests by the same investigator proved that *Ficus carica* L., *Areca catechu, Hibiscus esculentus* L., *Epiphyllum truncatum* Haw., and *Ricinus communis* are hosts to this fungus. Ocfemia (9) found the same organism in the Philip-

pine Islands to be very destructive to eggplant and, on inoculation, obtained positive proof of its pathogenicity on the bark of *Citrus maxima* Merrill (pomelo), *Capsicum annuum* L. (pepper), *Lycopersicum esculentum* Mill. (tomato), *Lactuca sativa* L. (lettuce), and *Solanum tuberosum* L. (potato). The senior writer found that *Allium cepa* is susceptible to root rot by all three organisms above mentioned.

Pethybridge and Lafferty (10) have suggested that *Ph. melongenae* and *Ph. terrestris* Sherb. be struck out as independent species and incorporated with *Ph. parasitica* Dastur. Godfrey (6) is in agreement with this. Ashby (2), taking the same point of view, suggested that *Ph. melongenae* and *Ph. allii* Sawada be incorporated with *Ph. parasitica*. *Ph. melongenae* doubtless constitutes a distinct variety of *Ph. parasitica* (3) if not a distinct species and the incorporation as suggested may not be a very wise one. If we consider *Ph. parasitica*, however, identical with *Ph. melongenae* then the number of hosts will be increased considerably. Ashby (1) found *Ph. parasitica* in Jamaica causing leaf-stalk rot of the cocoanut palm and two other closely related varieties of Phytophthora causing rots in tobacco and pineapples. Dastur (3, 4) has obtained his organism from castor beans (*Ricinus communis*) and from *Vinca rosea* L., where it was found first causing leaf-rot disease. Godfrey (6) found that *Ph. parasitica* var. *rhei*, an organism closely related to the pineapple parasites, causes root and crown rot of rhubarb (*Rheum rhaponticum* L.).

This brief summary of diseases caused by *Ph. Meadii* and *Ph. melongenae* indicates that the number of hosts may be greater than heretofore suspected.

PROPHYLAXIS AND CONTROL

Studies conducted for the control of this disease so far have given inconclusive results. The only recommendation, based mostly on observations made in areas where the disease occurs, is the adoption of the high-bed system of planting. This is effective in preventing flooding of the plants and decreases quite appreciably the chances of infection. Also, the proper draining of fields reduces the losses from this disease quite appreciably.

SUMMARY

The disease of pineapple plants in Hawaii known as heart rot or stem rot may be caused by any one of three different organisms, namely, *Phytophthora Meadii*, *Ph. melongenae*, or *Pseudopythium phytophthoron*.

The different organisms may invade the stem through its basal tissues, roots, or leaf axils, the ultimate result being the disintegration of the meristematic and juvenile tissues and leaf fall. Heavy rains, causing partial flooding of plants, favor the development of this disease.

Partial control of the disease may be obtained by planting in high beds to avoid flooding during very heavy rains.

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THE FUNGICIDAL ACTION OF ULTRA-VIOLET RADIATION

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It is the purpose of this paper to describe some recent work on the action of ultra-violet light on some specific fungi. At the time this work was initiated the writers were unaware that Fulton[2] had progressed so far in his experiments. Since, however, it is thought that some useful purpose will be served by outlining the work that we have recently carried out, the following resumé is here presented.

EXPERIMENTAL PROCEDURE

Source of light.—The lamp used was a quartz mercury-vapor lamp (Hanovia Artificial Alpine Sun, 2.5 amp. 210 v. direct-current type). The intensity of the light produced was roughly calibrated at intervals by means of the Erythema Dosimeter as devised by P. Keller.

Materials.—Potato agar was used as a medium for culturing the fungi that were selected for experiment. Eighteen petri dishes were used to contain this. The top cover was replaced, however, by a special form of glass, either Vita glass or Sanalux glass being used for this purpose. The medium was inoculated with the appropriate fungus from pure culture, a needle being used to introduce the organism. These dishes were irradiated for different periods and then compared with controls. The latter were similar cultures made in the same way but either with the ordinary petri dish cover or Sanalux or Vita glass for coverings. They were not irradiated.

Preliminary experiments.—The following fungi were selected for experiment: *Mucor Mucedo* L., *Rhizopus nigricans* Ehr., *Sporodinia grandis* Link, *Cladosporium herbarum* (Pers.) Link, *Dematium pullulans* de Bary and Löw, *Pleospora herbarum* (Pers.) Rabh., *Neurospora sitophila* Shear and Stev., *Fusarium* sp., *Sclerotinia trifoliorum* Eriks., and *Stereum purpureum* Pers. Cultures of these were made and on the same day radiation was commenced. In these preliminary experiments, they were irradiated for 9 minutes a day (this corresponds to 2 A.S.U.), the treatment being continued daily for a fortnight in the case of *Mucor Mucedo, Fusarium* sp. *Stereum pupureum,* and *Sclerotinia trifoliorum.* The difference between the irradiated plates and the controls was most marked. This is well illustrated in figure 1, A, B, C, and D, for Mucor and Fusarium. In both cases the mycelium has the appearance of "shrinking" away from the light. Actually, it does

[1] The writers are indebted to Mr. C. W. Williamson for the photographs of the plate cultures and to Mr. W. E. Dant for assistance in the preparation of material.

[2] Fulton, H. R. The fungicidal action of ultra-violet radiation. Jour. Agr. Research. 38: 159–168. 1929.

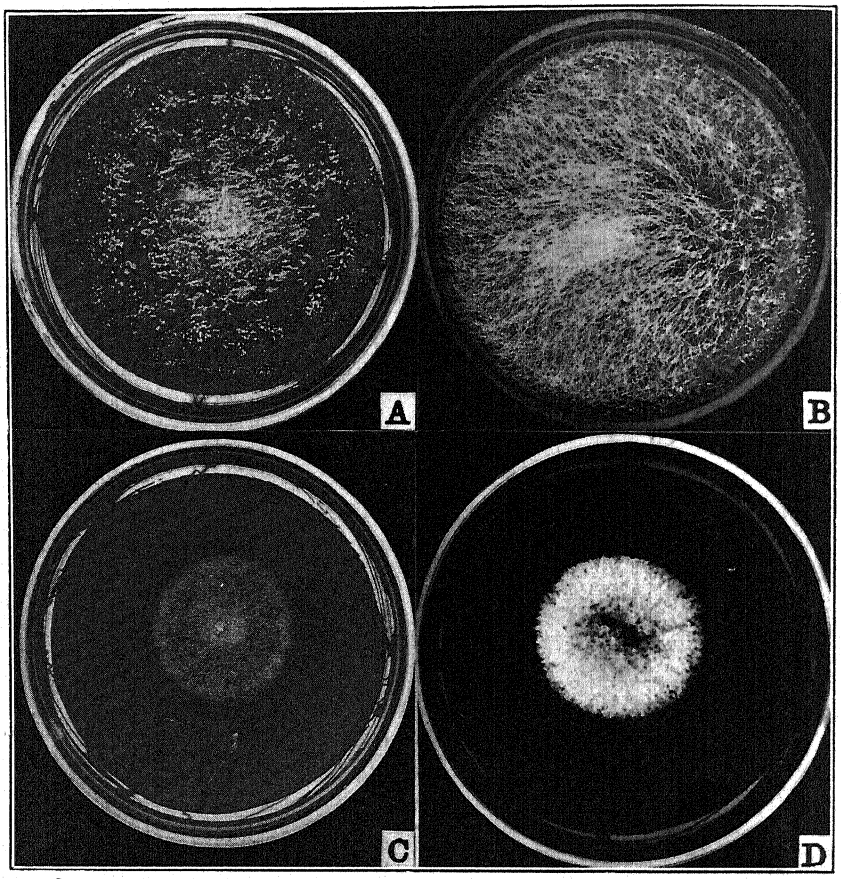


FIG. 1. A. *Mucor mucedo*. Radiated. B. *Mucor mucedo*. Not radiated. C. *Fusarium* sp. Radiated. D. *Fusarium* sp. Not radiated.

grow down farther into the medium, as if hiding from the light; consequently, aerial development is retarded and the sporangiophores and conidiophores appear stunted. After 7 days treatment these plates were reversed and treatment continued for another 7 days (*i.e.*, the plate receiving ultra-violet light was kept as the control and the control was illuminated). The effect of this treatment was to inhibit the growth of the original controls and to encourage growth in the cultures that had originally been retarded by the action of the light. This point is illustrated in figure 2, A, B, C, and D, the same plates as those shown in figure 1, A, B, C, and D, the difference of treatment being as stated, namely, that the former controls had been given light and the ultra-violet cultures had been kept as controls.

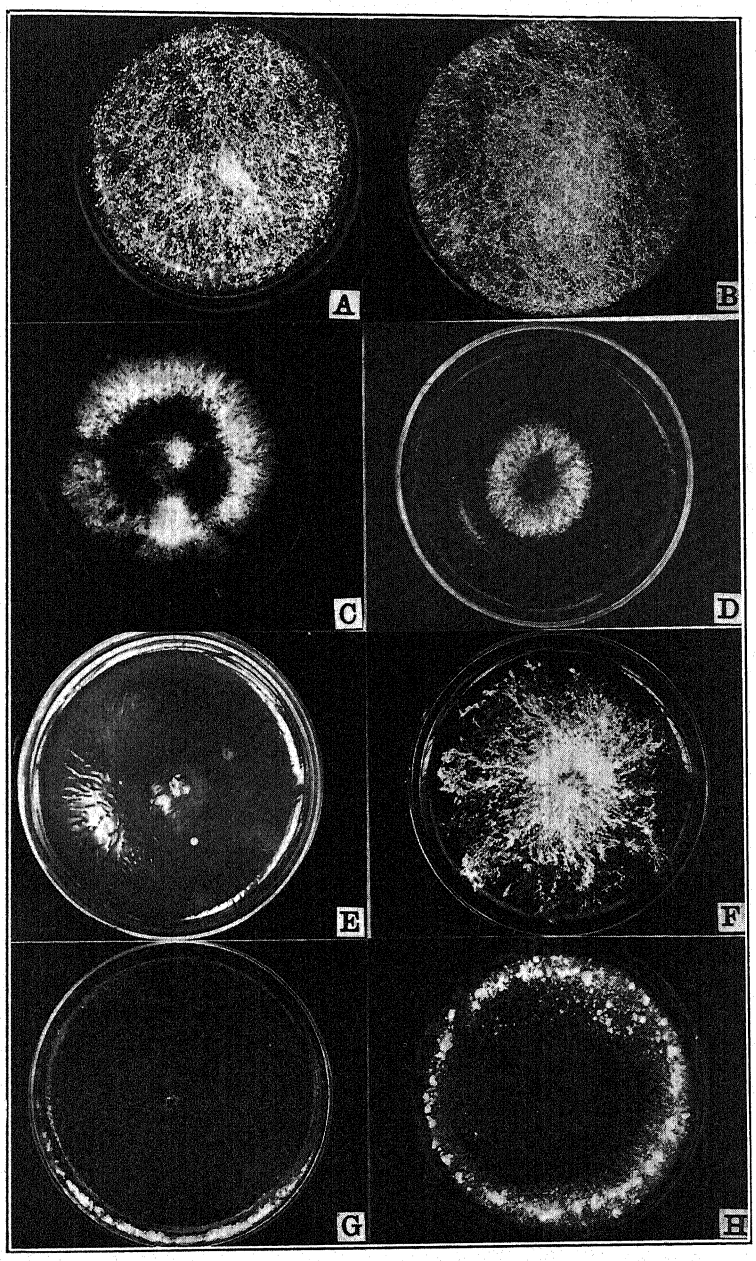


FIG. 2. A. *Mucor mucedo* (see Fig. 1, A). Following nonirradiation. B. *Mucor mucedo* (see Fig. 1, B). Following radiation. C. *Fusarium* sp. (see Fig. 1, C). Following nonirradiation. D. *Fusarium* sp. (see Fig. 1, D). Following radiation. E. *Stereum purpureum*. Radiated. F. *Stereum purpureum*. Not radiated. G. *Sclerotinia trifoliorum*. Radiation. H. *Sclerotinia trifoliorum*. No radiation.

This experiment suggests that in these cases the mycelium is not killed by the light but that growth is inhibited. Fulton,[3] on the other hand, asserts that the mycelium from germinated spores is more easily killed than the resting spores. Our results indicate the probability that in many cases the mycelium may not be killed but rendered dormant for some considerable time. Further experiments to be described will give more evidence on this point.

The effect of ultra-violet light given for 9 minutes per day for 14 days on *Stereum purpureum* was to check further growth and the inference then was that the mycelium had been killed. Figure 2, E and F, illustrates this point: Under similar conditions the rate of growth of *Sclerotinia trifoliorum* was checked, but at the end of the 14 days sclerotia were forming. With this fungus it was interesting to note that, when irradiated with ultra-violet light, the sclerotia were formed at the side of the dish, whereas in the control dishes they were formed much farther away from the side. In both fungi the mycelium grew well down into the medium away from the surfaces. Indeed, in all cases that were dealt with subsequently this was our experience. Figure 2, G and H, illustrates this phenomenon. The plates were photographed after 7 days.

With the other fungi that have been quoted similar results were obtained. Eventually, growth was checked and it was inferred that the mycelium had been killed. This was attributed to the fungicidal power of ultra-violet light. It was noted that the darker colored spores seemed to possess more resistance to light, thus confirming Fulton's observations.

In addition to these fungi, the action of ultra-violet light was tested on the chlamydospores of *Tilletia caries* Tul. produced on Chinese White wheat. The spores were placed on potato-agar medium in petri dishes; the plates intended for irradiation were covered with Sanalux glass. The controls had the normal petri dish cover. The controls were not irradiated, the others being given 9 minutes per day for a week. In the controls the majority of the bunt spores had formed primary conidia, in the case of those radiated; however, development had been checked and few primary conidia were observed.

To confirm the above general findings, 25 watch glasses were half filled with potato agar and then inoculated with *Mucor Mucedo*. A Vita glass plate was placed over these and four strips of carbon paper were pasted on the glass. The cultures were then irradiated for 9 minutes daily for a week with the result that where the watch glasses were covered with the strips of paper growth took place in a perfectly normal manner, but, where they were not covered, very little growth was apparent to the naked eye.

[3] *Loc. cit.*

At this stage preliminary experiments also were started on the action of ultra-violet rays on insects and other minute animals. Saprophytic eelworms from diseased Darwin tulips were placed in water in a watch glass. After one exposure of 9 minutes the majority of the adult eelworms were killed. In somewhat similar experiments the same results were obtained. Work in this direction is now progressing and the results will be communicated in due course.

EXPERIMENTS OF A MORE CONCLUSIVE CHARACTER

Nineteen petri dishes that had previously been exposed for spore-trapping experiments in the air, at altitudes varying from 500 to 13,000 feet, were taken. The contaminations of these were plotted and diagrams of the plates were made. The plates were then exposed for 15 minutes at one foot from the light. One exposure was made with the dish covers removed so that the light was cast directly on the naked culture. Subsequently the contaminations were again plotted and diagrams made when it was noted that the various colonies had not been killed but that more colonies had grown and pre-existing colonies were extending.

ACTION OF ULTRA-VIOLET LIGHT ON STEREUM PURPUREUM

Cultures of *Stereum purpureum* were made on potato agar in 8-cm. petri dishes with Sanalux glass tops. After a lapse of 6 days, in order to allow the fungus to establish itself on the medium, irradiation was commenced. Two series of cultures were irradiated daily, one series at 12 inches from the lamp, the other at 24 inches from the lamp. In each series separate cultures were exposed to the lamp at the following time intervals, 1 minute, 2 minutes, 4, 8, 16, 32, and 64 minutes, respectively. Radiation in this manner was given for 7 days, then the light treatment was stopped. From the records of growth made, it was observed that "the fungicidal action" of the light varied with the time given and the distance away from the lamp. This appeared to confirm Fulton's work; namely, "the killing effect of ultra-violet rays is increased with the lengthening of the time of action and also with the increase in the intensity of the radiation. Decrease in the distance from the source of radiation results in increase in intensity and in consequent killing effect."

In our own experiments we at first inferred that the mycelium had been killed. Subsequent examination 7 days later showed that in each case the fungus had grown and the rate of growth was in proportion to the time of exposure and the distance from the lamp. The mycelium had not been killed, but growth had been checked to the minimum and the fungus had grown away from the light and deep into the medium. Indeed, this could be observed in some cases at the bottom of the petri dish.

THE ACTION OF ULTRA-VIOLET LIGHT ON SCLEROTINIA TRIFOLIORUM

The experiment was designed in exactly the same way as the previous one, and the findings were the same. In this and the preceding experiment the control dishes with the ordinary covers had developed normally. For work of this description this fungus is an excellent one, as the rate of growth can easily be determined by the formation of sclerotia. In no case, however, was the mycelium killed. When light radiation ceased, growth was renewed and, as stated, the rate of new growth was in proportion to the time and distance of the previous exposure. It was again interesting to note that, when under the influence of light, the formation of sclerotia took place at the side of the dish.

DISCUSSION

When these results are compared with those obtained by Fulton the following points must be borne in mind.

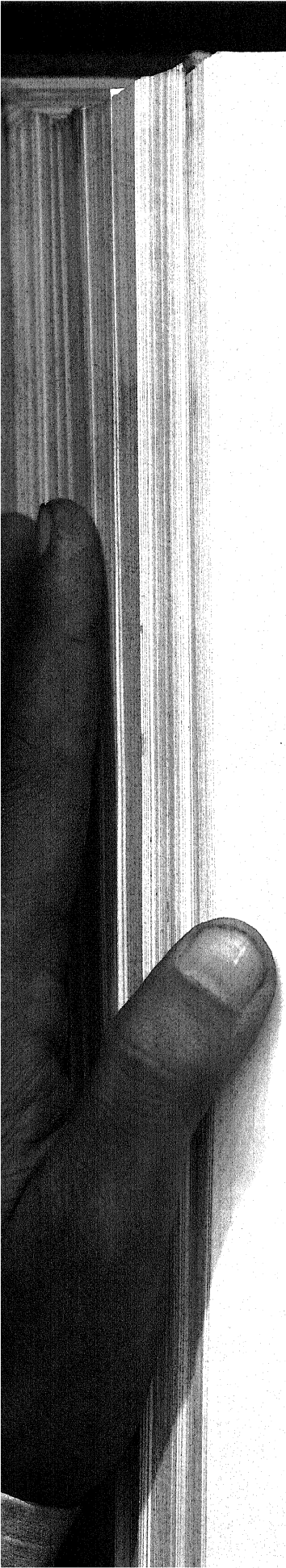
Fulton made his exposures with the glass tops removed; whereas, in these experiments Sanalux or Vita glass covers were used and these glasses transmit only 50 to 70 per cent of ultra-violet light. Again, Fulton inoculated his media by flooding the surface with spores or by blowing them on with an atomizer, *i.e.*, by surface inoculation only. In our experiments nearly all inoculations were made with a needle, so that spores and mycelium would be carried *into* the medium. Stevens[4] found that the minimum lethal dosage for exposed spores of *Glomerella cingulata* (Stonem.) S. and v. S. was somewhat above 10 but under 15 seconds, while, for spores covered by an agar layer about 15 mm. deep, the lethal period was over 90 seconds.

In our own experiments we at first thought the mycelium had been killed but, later, as we have shown, we realized that the mycelium was not dead but had been gradually checked in its growth and then rendered dormant. In all cases the mycelium grew deep into the medium to avoid the light. This is interesting since, in laboratory experiments that were carried out with *Mucor mucedo* grown in the normal way in petri dishes, the fungus grew towards the light. For example, a culture was made and a piece of carbon paper was pasted on the dish cover. A circular piece was cut from the middle of this disc. Later, it was found that the fungus had grown towards that portion not shaded by the paper. It is clear, therefore, that the different rays of light must have a different effect upon fungal growth.

[4] Stevens, F. L. Effects of ultra-violet rays on various fungi. Bot. Gaz. 86: 210–225. 1928.

It is here suggested that the higher fungi such as many of those in the Basidiomycetes—excluding the Uredinales and Ustilaginales—are intolerant of ultra-violet light and grow better when this is absent. It is an age-old superstition that "mushrooms," for example, grow only in the dark. These fructifications are formed more in the autumn, winter, and spring. It is generally agreed that growth of these and similar fructifications depends very largely upon moisture. It is, however, here further suggested that in damp and wet weather ultra-violet light is at its minimum and, consequently, growth is not inhibited.

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THE EFFECT OF BORON ON POWDERY MILDEW AND SPOT BLOTCH OF BARLEY

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The research results here reported show that boron may materially influence the susceptibility of barley to powdery mildew, *Erysiphe graminis,* and the spot-blotch disease, caused by *Helminthosporium sativum* P., K., and B. It has been frequently reported that the relative abundance of different soil elements or the character of a nutrient solution affects the development of plant pathogenes. Very little attention has been given, however, to the effect on parasitic fungi of those elements which in small amounts are now recognized as of great importance to the growth of higher plants. In view of the essential character of some of these elements and of the influence which they may have upon the development, structure, and composition of the host, it is perhaps to be expected that outstanding examples should be found in which the effects of such elements are extended to the parasites for which the host plant is the substratum.

Boron is now regarded as essential to many, if not all, of the higher plants, but it is likewise well established that this element may be toxic when supplied in relatively small amounts. The more sensitive plants are injured by concentrations as low as 3 or 4 p.p.m. in culture solutions, whereas other plants may not only tolerate but actually be benefited by concentrations as high as 15 or 20 p.p.m. A plant grown in a solution with a trace of boron supplied as an impurity with other chemicals may be expected to contain upward to perhaps 60 p.p.m. of boron in its dry tissue, whereas, with 15 to 25 p.p.m. in the culture solution, it may contain as little as 200 p.p.m. of boron or upward to 1,500 or even 2,500 p.p.m. The amount of boron found in plant tissue is relative not only to the variety of the plant but also to the stage of maturity and to the secondary environmental factors. The frequency of renewal of cultural solutions, containing boron only as an impurity from other chemicals, materially affects the amount of boron plants contain and, consequently, the severity of the symptoms resulting from boron deficiencies. These facts are mentioned for the purpose of indicating the diversity of conditions with respect to boron concentrations that a parasite may encounter within plant tissue.

EXPERIMENTAL METHODS

Barley, together with some 15 or 20 other plants, was grown in 18-inch rows in each of five beds of clean quartz sand. These beds, constructed of galvanized iron, were so arranged as to make it possible to flush and drain the sand daily with nutrient solutions. The nutrient solution employed

contained calcium nitrate, magnesium sulphate, and potassium acid phosphate in concentrations of 6, 3, and 3 millimoles per liter, respectively, and, in addition, $\frac{1}{2}$ p.p.m. of manganese as magnesium chloride. One ml. of a 5 per cent iron tartrate solution was added to each liter of culture solution. Boron as boric acid was added to this solution to give concentrations of 0, 5, 10, 15, and 25 p.p.m. in the different beds. The solution designated as 0 boron contained in the order of 0.02 p.p.m. of boron derived as an impurity from the C. P. chemicals used. After having reused the solutions for 7 or 8 successive days they were discarded and new solutions substituted.

California common barley and Onas wheat were grown in the summer of 1929 and again in the winter of 1929–30.

RESULTS

After having planted the summer crop of barley on May 22, a differential development of powdery mildew and spot blotch was noted in the different beds by the middle of June. This difference in the severity of attack became pronounced by the latter part of the month, and on July 4 specimen plants of both the wheat and barley were forwarded to Prof. W. W. Mackie at Berkeley for identification of the pathogenes and for such comment as to the severity of attack as the material would permit. Professor Mackie's reply was as follows:

"On my return from my annual vacation I found your letter of July 4, together with the specimens of barley and wheat grown in boron cultures. An inspection of the materials reveals the following:

Barley	Boron	0	p.p.m.	Mildew	4	Spot	Blotch	0
"	"	5	"	"	0	"	"	1
"	"	10	"	"	0	"	"	3
"	"	15	"	"	0	"	"	4
"	"	25	"	"	0	"	"	4

"The barley plants showed a very marked influence of boron upon the diseases. It seems from an inspection of the dried plants, which were not in a fresh condition, that spot blotch, *Helminthosporium sativum,* is fostered by boron in the plants because the specimens grown without boron in the culture showed no spot blotch, whereas all of the others showed rather heavy infestation. Mildew, which is common on all of our common barley, was found abundant upon the barley grown without boron, but entirely absent on those plants grown in the various boron solutions. It appears, therefore, that spot blotch is favored by boron, whereas mildew is entirely prevented in these specimens by the presence of boron.

"The wheat specimens, which were quite dry, appeared to have evidence of insect attack, but I was unable, owing to the condition of the specimens, to absolutely ascertain whether or not small traces of fungus diseases were present.

"The scale which I use varies from zero to 4, zero being no attack and 4 being maximum or heaviest attack of the fungus disease, as represented by the lesions."

The material sent to Professor Mackie was gathered during the hot part of the summer and it seemed possible that under more favorable conditions mildew might have developed on the barley, to at least a limited extent, in the cultures to which boron had been added. Boron concentrations in soil solutions as high as or higher than that in the 5 p.p.m. bed are not of uncommon occurrence under irrigation in California.

The effects of the boron treatments on the boron content of the plants and the combined effects of boron and the pathogenes on the dry weights of 8 plants of both wheat and barley are given in table 1. These plants were pulled from the beds on June 29 while yet quite immature. In agreement with the plant weights obtained from the later cropping of the summer-grown plants and from the subsequent winter plantings the growth of barley was depressed when boron was omitted from the culture solution, whereas no material reduction in weight occurred with wheat.

TABLE 1.—*Dry weights and boron content of 8 plants of barley and wheat grown in sand cultures from May 22 to June 29, 1930. Analyses by L. V. Wilcox*

Boron added to culture solutions, p.p.m.		0	5	10	15	25
Dry weight of plants, gms.	barley	16.3	20.8	16.2	12.7	11.0
" " " " "	wheat	10.6	11.1	8.3	8.2	6.2
Boron on dry weights, p.p.m.	barley	57	348	646	1045	1349
" " " " "	wheat	24	311	748	1058	1546

The winter plantings of barley and wheat were made on October 4, 1929. In agreement with the observations made on the summer planting, no evidence of the spot-blotch disease appeared on the barley plants in the 0 boron bed. If spot blotch was present it was so masked in its macroscopic appearance by the presence of mildew that it could not be identified as late as the middle of April. In the beds with 5, 10, 15, and 25 p.p.m. of boron, the spot blotch was abundant and of approximately equal severity under each of the four treatments.

In this winter planting of barley, powdery mildew was the most abundant on the plants receiving no boron, but it attacked plants in all of the

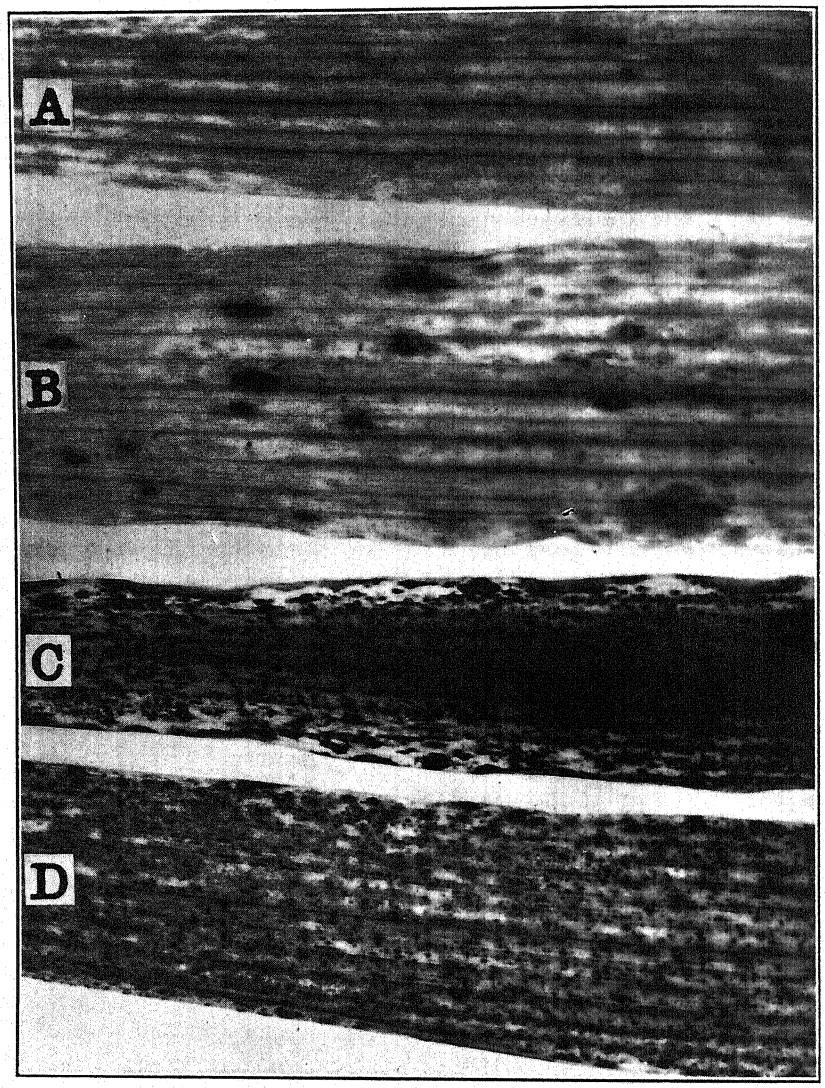


FIG. 1. Leaves of barley plants grown in sand cultures. A and B, from plants grown without boron and severely attached by powdery mildew, spot blotch being absent. C and D, from plants grown with 5 p.p.m. of boron. The latter were heavily attacked with spot blotch but some mildew also was present. B and D were the older and were largely devoid of chlorophyll. X 2½ approximately. March 28, 1930.

other beds to some extent. In the 0 boron bed the mildew was present on all leaves and many were almost covered. In the 5 p.p.m. bed it was also abundant, but the areas covered were smaller and they did not coalesce to the extent they did on the plants receiving no boron. Some leaves without mildew were found. Less mildew occurred on the 10 p.p.m. of boron plants than on those receiving the 5 p.p.m., and the amount was further reduced in the 15 p.p.m. of boron bed. In the 25 p.p.m. of boron bed only two well-shaded leaves on 100 culms were found infected. The differential development of powdery mildew and the spot-blotch disease on barley without boron is illustrated in figure 1.

Powdery mildew was present on the winter-wheat plants irrespective of the amount of boron supplied. In abundance it was approximately equal on all to that on the barley in the 5 p.p.m. bed.

A late winter planting of oats in the same beds was mildly attacked by mildew. Less mildew was present on the oat plants in the higher boron concentration, but the gradient was not so marked as that shown on the barley.

DISCUSSION

Although extreme specificity among the races of the different pathogenic fungi is quite common, it is nevertheless remarkable that the wheat and barley strains of powdery mildew, which attack only their own respective hosts and which in appearance are identical, should in the one case be so markedly influenced by the amount of boron supplied to the host plant and in the other almost uninfluenced by it. This difference in the reactions of the two strains of mildew seems especially noteworthy when it is observed (Table 1) that no material difference was found in the amount of boron in the dried wheat and barley plants.

The decreasing abundance of mildew on the barley as the boron supply was increased has made it appear most logical to place the burden of the interpretation of the foregoing relationship directly on the boron content of the barley plants. The fact that some mildew occurred on plants supplied with large amounts of boron, in the winter planting, and that mildew was abundant on the apparently normal barley supplied with 5 p.p.m. of boron would indicate that the development of mildew was not contingent upon structural abnormalities of the host plant.

Johnston and Dore[1] found an increased accumulation of sugar and starch in the leaves of boron-deficient tomato plants. If such accumulations of sugar and starch occurred in the barley they may have been contributing factors to the luxuriant development of the mildew in the bed with only a

[1] Johnston, Earl S., and W. H. Dore. The influence of boron on the chemical composition and growth of the tomato. Plant Physiol. 4: 31–62. 1929.

trace of boron. It should, however, be mentioned in this connection that Dufrénoy[2] found the plastids of chlorotic areas of citrus leaves affected with toxic quantities of boron gorged with starch. This observation might be taken to indicate that excessive amounts of boron may result also in accumulations of photosynthetic products in leaves.

The results clearly indicate that an amount of boron in barley plants greater than that supplied as impurity in the chemicals used is requisite for the development of the Helminthosporium disease.

SUMMARY

Barley and wheat were grown out of doors in large sands beds flooded daily with nutrient solutions. It was found that the spot blotch of barley, caused by *Helminthosporium sativum,* did not develop when boron was omitted from the culture solution. In a summer planting the abundance of spot blotch was successively increased by 5, 10, and 15 p.p.m. of boron, whereas in a winter planting the severity of the attack was approximately the same in these and the 25 p.p.m. concentrations.

In the summer planting of barley, powdery mildew, caused by *Erysiphe graminis,* was abundant on plants without boron and absent when boron was supplied. In the winter planting some mildew was present under all treatments, being abundant on the plants without boron and present on but two leaves of 100 culms grown with 25 p.p.m. of boron. No mildew developed on summer-grown wheat, but it was moderately and equally abundant on all winter plants, irrespective of the amount of boron supplied.

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[2] Dufrénoy, J. A cytological study of water soluble and fat soluble constituents of citrus. Jour. Agr. Res. 38: 411–429. 1929.

THE SUSCEPTIBILITY OF AMERICAN WHEAT VARIETIES RESISTANT TO TILLETIA TRITICI

I. REICHERT[1]

Those varieties of wheat, such as Ridit, Hussar, Martin and White Odessa, highly resistant to *Tilletia tritici* (Bjerk.) Wint. in the United States, have achieved, in addition to their practical value, considerable importance in elucidating the question of the occurrence of strains in bunt. Faris (1) was certainly the first to adduce experimental evidence of the existence of a difference in the virulence of bunt collections originating in different countries. However, definite light was thrown on this question only after the experiments of Sessous (10), Gaines (2), and especially of Roemer (8), who were the first to break down the exceptional resistance of the American wheat varieties with German collections of bunt and to show decisively the existence of different strains in bunt. Whereas the experiments of Faris (1) and of Stakman and Rodenhiser (6, 7) showed a slight difference in the infective capacity of the collections used, the works of Roemer (8), Reed (3), and Gaines (2), who worked with the above-named resistant varieties, demonstrated a complete difference in the virulence of the bunt collection ranging from entire capacity to entire incapacity for infection. These four bunt-free varieties were thus shown to be excellent indicators for the differentiation of strains of the bunt organism.

As we were experimenting with eight collections of bunt, used by Roemer (8) and Sampson (9), in an effort to infect wheat varieties that had proved to be bunt-free in Palestine (4, 5), we decided to try to infect the four highly resistant American varieties, using them as control plants.

Another object in introducing these resistant American varieties into our experiments was to investigate in Palestine the results obtained by various workers under different climatic conditions. In spite of the evidence adduced by previous investigators for the permanence of the bunt strains in different countries, there are some results contradicting that assumption and showing the marked influence of climatic conditions. To mention only one instance: The Halle-Saale, Germany, collection of *Tilletia tritici* was four times as virulent as the Pullman (Washington) collection on the variety Heils Dickkopf grown in Halle (8). On the other hand, the Halle collection was unable to infect the Heils Dickkopf variety grown at Pullman, whereas the Pullman collection produced 42 per cent infection

[1] The writer wishes to express his sincere thanks to Dr. T. Roemer, Halle-Saale, Germany, and Miss Kathleen Sampson, Aberystwyth, Wales, for their kindness in supplying him with wheat varieties and bunt collections for his experiments.

on Heils Dickkopf grown in Pullman (2). The experiments undertaken are therefore of general interest.

METHODS AND MATERIALS

We used the following four wheat varieties, Ridit, Martin, White Odessa, and Hussar, the first three varieties in two different stocks from Wales and Germany, respectively, making altogether seven stocks of wheat varieties, four of them, Ridit, Hussar, Martin, and White Odessa, provided by Dr. T. Roemer in Halle, Germany, and three varieties, Ridit, Martin, and White Odessa, procured by Miss Kathleen Sampson, in Aberystwyth, Wales.

The following *Tilletia tritici* collections were used: seven received by Dr. Roemer originating in different countries, one by Miss Sampson from Wales and one from Palestine—altogether, nine bunt collections.

The seeds were dusted in a container with *Tilletia tritici* spores taken from wild emmer plants, infected the previous year, and were sown in rows of 50 seeds on December 27, 1928. They were sown 15 cm. apart and spaced 7.5 cm. apart. The soil was sufficiently moist, the rainfall for the last ten days before sowing amounting to 33 mm. Rain fell again three days after sowing and the total amount during the next eight days reached about 80 cm. The temperature was thus reduced to 10° C. at the time of germination.

Many of the young plants, unfortunately, were destroyed by field mice, but the results from the remaining plants are sufficiently symptomatic and worth recording.

THE RESULTS

From the above results we see that the Ridit variety remained resistant to all bunt collections used, with the exception of the Breslau collection, which infected only the German stock up to 4.7 per cent. The high resistance of the Hussar variety was broken only by the Cosel bunt collection. Both stocks of the Martin variety remained immune from all the collections of bunt used. The White Odessa variety showed freedom from infection when inoculated with the Welsh, Pullman (in the Welsh stock), Wageningen, and Palestine bunt collections, slight susceptibility in the German stock to Pullman, and extreme susceptibility in both stocks to the Cosel, Breslau, and Landeskrona bunt collections.

The most virulent bunt collections were those from Cosel and Breslau (from 15 to 28 per cent). The weakest strains were from Palestine, Wales, Wageningen, and Zurich (not tried on all varieties). Pullman produced a slight degree of infection in the German stock of White Odessa, and Landeskrona caused heavy infection of White Odessa (on the German stock 5.2 per cent and on the Welsh 25 per cent).

TABLE 1.—*The percentage of bunted plants obtained in seven stocks of four varieties of wheat inoculated with spores of nine different collections of Tilletia tritici. Experiment conducted at the Experimental Farm, Gevath, Palestine, 1928–29*

Wheat varieties	Origin of the bunt collections								
	Aberystwyth, Wales	Pullman, U. S. A.	Cosel, Germany	Breslau, Germany	Wageningen, Holland	Landeskrona, Sweden	Lyngby, Denmark	Zurich, Switzerland	Gevath, Palestine
Ridit, Wales	0	0	0	.....	.....	0	.....	0	0
Ridit, Germany	0	0	0	4.7	.....	0	.....	0	0
Hussar	0	0	14.2	.....	0	0	.....	0	0
Martin, Wales	0	0	0	.....	0	0	.....	.....	0
Martin, Germany	0	0	0	.....	0	0	.....	.....	0
White Odessa, Wales	0	21.2	15.2	0	25.0	25.0	.....	.....	0
White Odessa, Germany	0	2.1	22.2	28.3	0	5.2	.....	.....	0

TABLE 2.—*Comparative behavior of eight collections of Tilletia tritici on Ridit, Hussar, Martin, and White Odessa in different countries*

Sources of inoculum	Wheat varieties and percentage of bunted heads											
	Ridit			Hussar			Martin			White Odessa		
	Amer. stock	Welsh stock	Germ. stock	Amer. stock	Welsh stock	Germ. stock	Amer. stock	Welsh stock	Germ. stock	Amer. stock	Welsh stock	Germ. stock
1. *Wales*												
a. in Wales	.....	21.0	.....	.....	4.0	.....	.....	0	.....	.....	6.6	.....
b. " Brooklyn	.....	.....	.....	0	.....	.....	0	.....	.....	0	.....	.....
c. " Palestine	.....	0	0	.....	.....	0	.....	0	0	.....	0	0
2. *Pullman*												
a. in Pullman	0	.....	.....	0	.....	.....	tr.	.....	.....	0	.....	.....
b. " Halle	.....	.....	0	.....	.....	1.0	.....	.....	1.0	.....	.....	8.0
c. " Brooklyn	.....	.....	.....	0	.....	.....	0	.....	.....	0	.....	.....
d. " Palestine	.....	0	0	.....	.....	0	.....	0	0	.....	0	2.1
3. *Cosel*												
a. in Halle	.....	.....	7	.....	.....	50.0	.....	.....	77	.....	.....	83.0
b. " Palestine	.....	0	0	.....	.....	14.2	.....	0	0	.....	21.2	22.2
4. *Breslau*												
a. in Halle	.....	.....	10	.....	.....	46.0	.....	.....	61.0	.....	.....	85.0
b. " Palestine	.....	.....	4.7	.....	.....	.....	.....	.....	.....	.....	15.2	28.3
5. *Wageningen*												
a. in Halle	.....	.....	6.0	.....	.....	0	.....	.....	1	.....	.....	7.0
b. " Palestine	.....	.....	.....	.....	.....	0	.....	0	0	.....	0	0
6. *Landeskrona*												
a. in Halle	.....	.....	1	.....	.....	1	.....	.....	3	.....	.....	2
b. " Palestine	.....	0	0	.....	.....	0	.....	0	0	.....	25.0	5.2
7. *Lyngby*												
a. in Halle	.....	.....	2	.....	.....	19	.....	.....	20	.....	.....	16.0
b. " Palestine	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8. *Zurich*												
a. in Halle	.....	.....	0	.....	.....	0	.....	.....	12.0	.....	.....	4.0
b. " Palestine	.....	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....

A comparison with the results of other countries.—It may be interesting to compare the results obtained in the Palestine experiments with the outcome of similar work in other countries, especially with those of Roemer (8), who used the same wheat varieties and seven bunt collections, as we did, and of Reed (3), who used three of our wheat varieties and two of our bunt collections; and, further, with that of Miss Sampson (9), who used all four wheat varieties and one of our bunt collections.

The above table gives a clear survey of the infective capacity of the eight bunt collections on different stocks of the four American wheat varieties grown under different climatic conditions.

We see that the Welsh bunt infected Ridit in Wales but not in Palestine, even from stock originating in Wales. It was able also to produce slight infection on Hussar in Wales but not at Brooklyn or in Palestine. It behaved similarly on White Odessa. The Martin variety remained immune from the collection in all three countries.

The Pullman bunt was unable to produce infection on Ridit at either Pullman or Halle or in Palestine, where experiments were conducted. When inoculated with the Pullman strain Hussar remained in Pullman, Brooklyn, and Palestine, and was only lightly infected in Halle. The Martin variety also showed a high degree of resistance to the Pullman bunt. In the Brooklyn and Palestine experiments no infection occurred, and in the Pullman and German trials only a very slight amount of infection developed. The American stock of White Odessa was not infected either in Pullman or in Brooklyn. The German stock, however, showed a fair amount of infection both in Germany and in Palestine.

The Cosel bunt collection infected Ridit and Martin in Halle but not in Palestine. It also caused heavy infection in the German stock of Hussar in Halle and in Palestine. Both stocks of White Odessa were severely infected by this collection in both Germany and Palestine.

The Breslau bunt collection gave the same positive results in Germany as in Palestine. Both stocks of White Odessa were heavily infected by this collection in both countries—Germany and Palestine.

The Wageningen bunt collection was unable to infect Hussar either in Germany or in Palestine. Martin and White Odessa were infected by the bunt collected in Germany but, in Palestine, not even the German stock contracted the disease.

The Landeskrona bunt failed to infect Ridit, Hussar, and Martin in Palestine but caused slight infection in Halle, Germany. White Odessa was infected by this collection in Germany as well as in Palestine, more virulently in the latter than in the former country.

The Zurich bunt collection caused no infection on Ridit, either in Palestine or in Germany.

Number of strains.—An attempt may now be made to summarize the differential reaction of the four wheat varieties to the nine collections of bunt, as shown by the results in the different countries. They are more likely to be correct, as they are based on the results obtained under different climatic conditions.

TABLE 3.—*Number of bunt strains based on the susceptibility of Ridit, Hussar, Martin, and White Odessa to nine collections on bunt in different countries*

No. of strain	Name of coll.	Name of the wheat varieties and their + or − susceptibility			
		Ridit	Hussar	Martin	White Odessa
1	Wales	+	+	−	+
2	Pullman	−	+	+	+
3	Cosel	+	+	+	+
	a. Breslau				
	b. Landeskrona				
	c. Lyngby				
4	Wageningen	+	−	+	+
5	Zurich	−	−	+	+
6	Palestine	−	−	−	−

From the above table it is evident that six bunt strains may be differentiated among the nine on the basis of the behavior of the four wheat varieties. Reed (3), who used 21 collections of bunt and five wheat varieties, was able to distinguish only six strains. His strains are not identical with those shown in the above table. It is evident that many more than six strains are known today. The Palestine collection represents a special strain, as shown elsewhere (5).

CONCLUSION

On the whole, the experiments carried out in Palestine confirmed the results obtained by Roemer (8). The bunt collections of Breslau and Cosel, which proved the most virulent in the experiments in Halle (Germany), also maintained their infective capacity in Palestine under quite different climatic conditions. The Welsh, Pullman, Landeskrona, and Zurich collections gave almost identical results in both countries, Germany and Palestine. The Landeskrona collection seemed to have lost some of the virulence it manifested in Germany. A greater loss of virulence was observed in the Cosel bunt when applied to the Ridit and Martin varieties. Very striking is the change in the Welsh bunt, which lost its infective capacity for Ridit,

Hussar, and White Odessa in Wales when employed in the United States (Brooklyn) and in Palestine.

Hardly any difference in reaction to the bunt of the different collections was noticed between the different stocks of the wheat varieties used in Palestine. Only in one out of 16 experiments with both stocks was a slight difference noticed in White Odessa. This is not quite in agreement with Stadler's findings (11).

Such modifications in the virulence of the bunt of certain collections in other countries are difficult to explain. Temperature influence seems to play no part, as an optimum of infection occurred in all experiments. More plausible, perhaps, is the assumption that other climatic conditions are the decisive factors in the change. It must be assumed that these conditions inhibit the germination of the more virulent components of the bunt collection and favor the development of the less virulent ones. Furthermore, the possibility of an alteration in the capacity of the host for resistance should not be excluded, but this hypothesis is considered less probable.

SUMMARY

1. Experiments were carried out to determine the behavior of nine bunt collections towards the Ridit, Hussar, Martin, and White Odessa wheat varieties.

2. On the whole, the results obtained confirmed those of Roemer. The Breslau and Cosel bunt collections proved to be the most virulent and those of Wageningen, Zurich, and Palestine the weakest.

3. The permanence of the virulence in different countries with different climatic conditions was then proved. It must, however, be admitted that modifications are also possible since they occurred in the Welsh bunt collection.

4. Six different strains of bunt were differentiated in the collections used on the basis of their behavior towards the four wheat varieties.

TEL-AVIV,
PALESTINE.

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STRIPE RUST, PUCCINIA GLUMARUM, ON WHEAT IN ARGENTINA

HARRY B. HUMPHREY[1] AND RICHARD O. CROMWELL[2]

Stripe rust, *Puccinia glumarum* (Schm.) Eriks. and Henn., in South America, was first collected there by E. W. D. Holway, in 1919 (1) who found it on *Hordeum chilense* R. and S. at Viña del Mar, Chile. In 1920 he found it at Riobamba, Ecuador, on *Agropyron attenuatum* R. and S. Elsewhere, in South America, it apparently had never been collected until October, 1929, when the junior writer observed it in epiphytotic proportions on wheat in Argentina. He is under the impression that he saw it there in 1926, also, but made neither a collection nor a record of it. While in Argentina in 1929 he had opportunity to make extensive observations on the occurrence and severity of the epiphytotic. Accordingly, a detailed report of his findings was prepared with a view to its ultimate use in the preparation by the senior writer of a somewhat more ample account of stripe rust in the Americas.

Stripe rust has long been recognized as one of the most important cereal diseases known to European agriculture. Its ravages are most severely felt by the farmer who depends upon wheat for a portion or for all of his income. In the United States this rust is, thus far, known to occur only in the Pacific and Intermountain States. Potentially, it is tremendously important to the great wheat-growing regions of the Mississippi Valley and the Eastern States where, by chance, it may some day be introduced through transportation of crop products or otherwise from parts of the world subject to stripe-rust epiphytotics.

The occurrence of stripe rust in the Western Hemisphere was discovered on wheat in Arizona by F. Kølpin Ravn (4), in 1915, while touring the United States as a guest of the Department of Agriculture. Further research by the senior writer *et al.* (5) disclosed the fact that this rust had first been collected in the United States by Piper, in 1892, on *Elymus glaucus* Buckl. and *Bromus carinatus hookerianus* (Thurb.) Shear. It was later collected by E. and E. T. Bartholomew on *Sitanion hystrix* (Nutt.) J. G. Smith. These early collections of *Puccinia glumarum* were, however, not recognized as stripe rust but were assigned to *P. agropyri* and *P. rubigo-vera* where they remained until 1922, when they were correctly identified by the senior writer. In 1896 it was collected by Holway on *Hordeum jubatum* L., near Mexico City, Mex., and assigned to the species *P. rubigo-*

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vera. None of these early collectors recorded the occurrence of this rust or made any collections of it on wheat or other cultivated host.

Since its discovery in North America by Ravn stripe rust has been collected widely throughout the Pacific and Intermountain States, excepting New Mexico and Nevada, and was observed in August, 1919, as far east as Newell, S. Dak. In western Canada it is more or less prevalent on wheat and certain congenial grass hosts in Vancouver Island and elsewhere in British Columbia, and to a limited extent in Alberta and western Saskatchewan. Stripe rust occurs rather commonly and sometimes devastatingly in Mexico, where it has been observed in the Yaqui Valley and certain other important wheat areas as far north and east as Monterey. Its presence in Mexico and apparent absence from Texas have puzzled us not a little in attempting to account for the possible Mexican source of certain physiologic forms of *P. graminis tritici* Eriks. and Henn. Its failure to establish itself in near-by Texas may possibly be explained in part by the fact that the prevailing winds that move across the northern half of Mexico during April, May, and June, according to Bartholomew and Herbertson (2) and the more recent unpublished studies of the senior writer, blow from the southeast and east to northwest rather than from the south. This fact also may in part account for the general prevalence of *P. glumarum* in Arizona and California.

The late Dr. Carlos Spegazzini of La Plata, Argentina, collected extensively and with more than usual thoroughness the rusts and other fungi of Argentina over a period of 42 years, or from 1880 to 1922. During that time he published 19 papers, or a total of nearly 2,500 pages; yet, not once does he record the occurrence of *P. glumarum* in Argentina. Other collectors and students of the rusts of South America, such as Gustav Gassner, P. Hennings, E. Ule, and others, failed either to observe or record the presence of stripe rust east of the Andes. Arthur (1) states that there are certain collections recorded under the names *P. rubigo-vera, P. sessilis, P. straminis,* etc., that he has not yet seen. The examination of these and other South American collections may yet disclose the presence of *P. glumarum* beyond the confines of Ecuador and Chile.

The junior writer's 1929 observations on the conditions affecting the wheat crop of Argentina covered approximately 80 per cent of the total wheat acreage. His field inspections began near the northern limits of the main wheat belt, in the Provinces of Santa Fé and Cordoba, in October, and progressed southward to near the southern limits of the belt, in southern Buenos Aires Province and Territory of Pampa, through November. This allowed each locality to be observed while the wheat was in the blossom-to-milk stage of development.

The severe epiphytotic of stripe rust in the territory covered was confined roughly to the area encompassed by Salliquelo, Colonel Suarez, Tres Arroyos, Necochea, and Tandil in the southern part of the Province of Buenos Aires and comprised about 40,000 square miles. East of the above area to the coast was not inspected because the wheat acreage is extremely light there. For the same reason an area east of a line—Buenos Aires City to Bragado, Bolivar, La Madrid, Rauch, and Maypu—was not seen. From reports, however, it was assumed that stripe-rust infection was moderate to severe wherever wheat was grown in either of these eastern areas. As one proceeded to the north or west from the seacoast the amount of this rust diminished until it became light or none at the northern and western borders of the Province and beyond. There was a trace of it in parts of southern Santa Fé. Likewise, drouth greatly affected wheat in the remainder of this Province. Cordoba's wheat crop was almost a failure from drouth, and the Province was visited under such conditions that it is impossible to report more definitely than to state that the disease was nearly, if not quite, absent. The extreme west-central part of the Province of Buenos Aires, around Maza, Rivera, Puan, Alta Vista, Villa Iris, and especially west thereof, was scarcely affected, for the early-season drouth resulted in a poor plant growth. In this region, where Kanred is popular, the average yield approximated two bushels per acre. At the new Southern Railway experimental farm, Bordenave, Buenos Aires, a trace of stripe rust was seen.

In the Tres Arroyos territory there were two optimum periods for the development of *P. glumarum*. A heavy infection killed the lower leaves during the boot-to-blossom stage of the host. Then the rust seemed for a time inactive only later to resume the attack and kill the remaining leaves during the milk stage of kernel development. This final attack was severe also on the glumes, awns, and kernels of susceptible varieties. Markedly noticeable differences in what may have been varietal susceptibility were observed in several varieties.

The weather at Tres Arroyos during the life of the 1929 crop is of interest because the stripe-rust epiphytotic was as severe there as anywhere within the area visited. Precipitation for 1928 is given but a record of the temperatures for that year was not available. In the Tres Arroyos district wheat is sown from late May to early August and harvested in December and January.

Table 1 shows that the October precipitation was 75 millimeters in excess of normal. It was near the end of October and the beginning of November when the epiphytotic of *P. glumarum* appeared. Temperatures had not been unfavorable. Early wheat was beginning to head about Octo-

TABLE 1.—*Monthly precipitation for 1928, precipitation and maximum and minimum temperature for the first 11 months of 1929, and the normal average and actual average temperature for the period of June to November, 1929, inclusive, at Tres Arroyos, Province of Buenos Aires*[a]

Month	Precipitation in millimeters			Temperature (degrees C.)			
				1929			
	1928	Normal	1929	Max.	Norm. av.	Actual av.	Min.
Jan.	10.5	65.0	66.0	38			4
Feb.	161.0	74.0	92.5	37			5
Mar.	34.0	68.0	18.0	35			1
Apr.	177.0	36.0	73.0	30			0
May	48.0	32.0	53.5	21			-1
June	20.5	35.0	45.0	17	8.2	8.0	-3
July	41.0	40.0	7.0	20	7.7	8.1	-3
Aug.	2.0	33.0	15.0	21	8.7	9.2	-4
Sept.	5.0	51.0	55.0	28	11.0	10.8	-3
Oct.	58.5	63.0	138.0	36	13.7	12.5	-3
Nov.	55.0	58.0	41.0	38	17.0	18.8	4
Dec.	8.0	72.0	.....	.....	.....	.....	.....
Total ...	620.5	627.0	604.0				

[a] Jan. to June are Experimental Farm unofficial figures except normals, which are official. July to Dec. are official.

ber 21. Four weeks of favorable rust weather was sufficient to bring about the full natural development of a severe rust epiphytotic, and the abundant rainfall of October undoubtedly promoted its development during the critical period of the wheat plant's growth. The records have been searched to learn in what other years the precipitation in October and November exceeded the normal to an approximate degree, and it has been found, as indicated in table 2, that the moisture and temperature conditions for the period in question were sufficiently favorable in 1920 and 1922 to have produced an epiphytotic of stripe rust provided enough inoculum had survived the rigors of the preceding winter months. There is, however, no record of the presence of this rust in Argentina in those years.

Other factors necessary to an outbreak of *P. glumarum* on winter wheat (6) include conditions favorable to infection and spread during the fall (late April, May, June, in southern Buenos Aires) and early spring (end of August and September). At Tres Arroyos there was sufficient rainfall, apparently, to favor spread of stripe rust while wheat was young in the fall and subsequent early spring. The year 1928 produced a good crop with autumn moisture comparable to that of 1929, scant in early spring, and normal thereafter. There was no noticeable rust damage of any kind.

TABLE 2.—*Monthly precipitation and mean monthly temperature for the years 1920 and 1922, respectively, at Tres Arroyos, Province of Buenos Aires*

Month	Precipitation in millimeters		Temperature (degrees C.)			
			1920		1922	
	1920	1922	Mean max.	Mean min.	Mean max.	Mean min.
Jan.	42	143	31.1	13.6	31.0	14.4
Feb.	66	100	29.2	13.0	27.8	12.1
Mar.	40	43	28.2	13.4	26.9	11.9
Apr.	112	54	23.3	10.0	21.3	7.4
May	2	23	17.9	6.3	17.7	6.0
June	5	125	13.0	1.0	10.4	2.8
July	48	26	12.1	0.8	14.3	6.0
Aug.	5	19	17.0	2.6	14.2	2.6
Sept.	71	21	18.7	4.9	19.1	4.7
Oct.	107	94	18.7	7.7	17.5	6.4
Nov.	67	146	23.8	10.0	25.3	10.9
Dec.	202	60	27.8	12.6	30.4	12.2
Total	767	854				

Argentina's better-seed campaign probably has imported more lots of seed wheat since 1925 than at any time in the last three decades. These imports increased annually during the period 1925 to 1928. Government and railroad multiplication and distribution of these imports and of varieties bred locally by 1929 brought about almost a complete change of varieties grown throughout the nation from those grown in 1925. In view of this fact there is a possibility, if not probability, that the sporadic outbreak of stripe rust in Argentina may have had its origin through spores brought in on plant material in shipments of wheat received from European sources. Hungerford (6) has shown that the urediniospores of this rust retain their germinability two to three months under normal conditions, and Becker (3) has established the fact that under conditions of refrigeration they are germinable after 430 days.

The potential importance of stripe rust to cereal culture in the major wheat-producing areas of the Americas is so great as to warrant a thorough and comprehensive investigation of the problem. Much as has already been contributed to our knowledge of this rust there yet remains a great deal to be learned about its epiphytology, its possible methods of introduction into new areas, its physiologic specialization, life history, and control.

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FROST-TOLERANT AND BLIGHT-RESISTANT POTATOES

DONALD REDDICK

FROST TOLERANCE

In such a warm, dry season as existed in most of the northern potato regions during the summer of 1929, the potato makes very little growth until the rainy, cool days of September arrive. It is in such years that early frost is most unwelcome. Ten days or two weeks of growth under favorable conditions may make an enormous difference in the yield of No. 1 tubers. Likewise, a January frost in the Southern States may injure the young plants and thus delay the harvest. In 1929, in New York, killing frost occurred on September 20 and 21 throughout most of the potato sections, but this was followed very generally by fourteen to twenty days of weather very favorable for tuber production. At Ithaca the official record of the U. S. Weather Bureau for each of these nights was 29° F. (−2° C.). Unfortunately, there were no thermometers present in the potato plot in which the following observations were made on these nights. The plot is about one mile distant from the recording station and usually runs about 1° F. colder at night than the official record.

It was not surprising to find in the experimental plot some plants that had withstood with no injury whatever two successive nights of 29° or slightly colder, because there were present in the field plants of *Solanum demissum* Lindl., seeds of which were kindly supplied to me by Dr. C. F. Clark. *S. demissum* is reported as an herbaceous perennial from the mountains of Mexico at 8,000 to 9,000 feet (Uhde). From his recent paper it is clear that Salaman (9)[1] is concerned with this same species although he uses the later name *utile* Klotzsch. Rydberg (8) regards the two as identical and even Bitter (1),[2] regards the two as very close and suggests that further study may show them to be identical. *S. utile* is likewise reported from Mexico (Rio Frio, at about 10,000 feet, and from Michoacan, at about 8,000 feet). It is even possible that the original material on which the species is founded is identical. The same collector is cited, Mr. C. A. Uhde, "a German gentleman, who has resided for many years in the west of Mexico," and the same general locality is also recorded. Lindley (6) quotes as follows: "Native Mexican potatoes, growing at 8000 to 9000 feet elevation" while Klotzsch (4) secured seeds from the director of the Berlin Garden, Fr. Otto, with the legend: "wilde Kartoffel aus Rio Frio im Hochgebirge zwischen Puebla und Mexico, in einer Höhe von 10,000 Fuss über den Meerspiegel vorkommend."

[1] L. c., p. 329.

[2] L. c., p. 454.

The report of many travelers in Mexico and in the higher Andes of Peru and Bolivia is that in those regions where "wild potatoes" are found the night temperatures are very low and that light frosts may occur in almost any month of the year. It was to be expected that a species from such a region would show some frost tolerance.

The behavior of first-generation hybrids with *demissum* is interesting. Such hybrids have not been easy to secure and only a small number of plants were in the test, but, without exception, they withstood the two successive cold nights without injury. The hybrids and the number of seedling plants involved are: *demissum* x *maglia*, 7 plants; *demissum* x *fendlerii*, 13 plants, (a few terminal leaves showed very slight injury). Both *maglia* and *fendlerii* in the immediate vicinity were killed to the ground.

A wholly unexpected case of frost tolerance, however, appeared in a culture of *S. commersonii* Dunal and its hybrids. It adds another example to White's (10) list of warm-climate frost-tolerant plants. A very generous sample of seed was secured from the Director of the Botanical Garden in Montevideo under the name *commersonii*. The plants grown from this seed were all very uniform in appearance but differed in several minor particulars from *commersonii* secured from J. R. Baiz, Las Delicias, Argentina, or *commersonii blanca* from the same source, or from *commersonii* forma *silvestre* secured from J. B. Marchionato, La Plata, Argentina. This species presumably is limited to the coastal-plain region and, while it may be inferred that its ancestral home was in the hot equatorial region and that its present geographical distribution indicates a line of development significant of greater tolerance for cold, there seemingly is nothing in its present distribution to indicate a tolerance for below-freezing temperatures. This particular lot of *commersonii*, however, showed no injury whatever from the two freezes, although the three other families carried under this name were killed completely. Likewise first-generation hybrids with the *commersonii* from Montevideo exhibited a similar tolerance, whereas, such hybrids with *commersonii* from other sources were killed completely. Here again the number of plants involved is very small because of difficulty in effecting crosses. The hybrids obtained and the number of seedling plants on which the observations are based are as follows: *commersonii* (Montevideo) x *commersonii* (Las Delicias) 2 plants; *commersonii* (Montevideo) x *demissum* 12 plants. In the latter case the frost tolerance might be ascribed equally as well to the pollen parent. No records exist for hybrids in which the pollen was taken from *demissum* although, as will be mentioned below, Klotzsch found tolerance with the reciprocal. In the case recorded here, the plants involved are unquestionably hybrid because they resemble the pollen parent much more than they do the mother.

Some other plants in the experimental garden exhibited frost resistance. These were found among some varieties secured from Professor Knappe, in Estonia. 1. *S. commersonii* (Montevideo), doubtless from the same source as the plants mentioned above. 2. Caliban (*demissum* x Majestic). 3. Silhonetto (Sylvan x Hindenburg). 4. Also in some varieties secured from Dr. R. Schick, Müncheberg, Germany, to test for resistance to late blight (*Phytophthora infestans*), Wohltmann, Parnassia and Deodara proved to be not only very resistant to blight but also resistant to frost. Many other varieties were grown in the same field and those that had survived the blight were completely killed by the low temperature of September 20. Some of the varieties involved were Green Mountain, Russet Rural, Evergreen, Ekishirazu, and a long list of hybrids several of which were from Professor Knappe.

It is worthy of more than passing note that three German varieties out of a total of five included in the test should exhibit resistance to Phytophthora blight and tolerance for cold. Doctor Klotzsch (5) hoped that his *utile* would prove useful in combating "the potato disease." The hope of accomplishing this seems to have been great, for, a few weeks later, in the same publication (p. 356), the statement is found that Juhlke (3), gardener at Akademie Eldena, was growing nearly a half acre of seedlings of this species. This article usually is cited as authority for the susceptibility of this species to *P. infestans* and the article is commonly ascribed to Münter. Juhlke is responsible for the communication to the editor and Münter is quoted by him as saying that this ought to bring to an end the discussion about the potato disease being a degeneration caused by continued asexual propagation.

Whatever effect this announcement may have had on Klotzsch, it did not deter him from continuing work with the species. In 1851 he (5) says nothing whatever about the potato disease, but he reports that *utile* was not injured by frost of 2° (Réaum.) under the freezing point. He reports further that in the hope of introducing this frost tolerance into the ordinary varieties he had placed the pollen of *"tuberosum"* on *utile,* had secured a blend of the two and had grown the seedlings. At the end of March, half the seedlings were grown in pots out of doors where they were subjected several times to frost of 1½° (Réaum.) without injury.

BLIGHT RESISTANCE

The susceptibility of *S. demissum* to blight is recorded by Lindley in his original paper (6). With the great prevalence of blight at the time and the intense interest in the disease it would seem that no mistake could have been made either by Klotzsch or Lindley. It must be remembered,

however, that at that time *P. infestans* was not commonly regarded as the cause of blight but merely an invader of dead tissue. Its presence was not a diagnostic character. *S. demissum*, as grown at Ithaca, often shows a marginal burning of the leaves and occasionally this has become so severe that certain plants died completely. The cause of this burning is not known. It has much the appearance of blight as it occurs on the variety Rural New Yorker but *P. infestans* is not present. In fact, repeated attempts to infect this species artificially with *P. infestans* under thoroughly favorable conditions both with very small seedlings and mature plants have consistently failed. No lesion whatever is formed. Salaman (9) has grown the species, under the name *utile*, for 15 years and it has always been free from blight. It seems probable that Lindley and Juhlke were mistaken about the susceptibility of the species.

An attempt to relate the varieties Deodara, Parnassia and Wohltmann to Klotzsch's *utile* seems reasonable on the basis of two outstanding physiological characters. The immediate parents of these varieties are known. The first two are sibs of Deutsches Reich by Jubel. The latter is the offspring of Daber by Erste von Frömsdorf. An examination of all of the known treatises on systematics of the potato fails to reveal the lineage through many generations. None of the varieties mentioned in the lineage, so far as it can be traced, can be very old, for none is found in the long list of varieties (2) exhibited at Altenburg in 1875. Incidentally it is interesting and perhaps significant that in this list, mostly of varieties with German names, the exotic name "Rio Frio" should be attached to one of the old and well-tried (altbewährten Sorten) varieties and that among the novelties a variety was exhibited under the name "Dr. Klotzsch Bastardzucker."

All of the first generation hybrids of *demissum* mentioned above likewise exhibited complete immunity from *Phytophthora infestans*, both in greenhouse inoculation and in the field. Natural infection was very general in the experimental plot on susceptible sorts. An initial artificial inoculation had been made on a susceptible variety planted for the purpose. Subsequent weather conditions favored a natural spread of the disease. All of the plants of *demissum* and its hybrids had been grown from seeds. The seeds were planted in mid-April and the seedlings were transplanted to the field early in June. Seeds and tubers had formed at digging time (October 10) and the plants may be said to have matured even though they remained green. The inoculations made in the greenhouse were done in March, 1930, and a more extensive report will be presented later. The contention of K. O. Müller (7) that plants become susceptible to Phytophthora when they approach maturity is not supported by these observa-

tions, but, obviously, much more extensive tests must be made either to confirm or to deny his claim.

SUMMARY

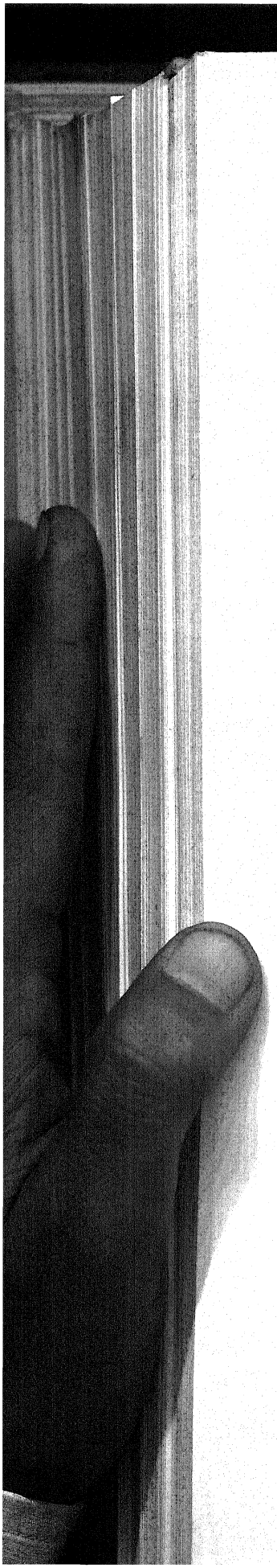
Solanum demissum and *S. commersonii* from Montevideo were subjected on two successive nights to 29° F. without any apparent injury. Some first-generation hybrids of each of these species showed a similar frost tolerance. *S. commersonii* from three other sources, along with numerous varieties of *S. "tuberosum,"* were killed completely by the cold.

Solanum demissum is immune from *Phytophthora infestans* as are also some first-generation hybrids.

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GERMINATION OF THE OOSPORES OF SCLEROSPORA GRAMINICOLA (SACC.) SCHROET.[1]

M. M. Evans and George Harrar[2]

Comparatively little is known about the germination and function of the oospores of the Peronosporales. Most of our knowledge of the germination of oospores and their function goes back to de Bary's work. He has described the germination of the oospores of *Peronospora valerianellae* Fckl. (3), *Cystopus candidus* Lev. (1), and *Pythium debaryanum* Hesse (2). Moreover, he reports that the resting spores of *Peronospora valerianellae* and *Pythium debaryanum* germinate by a tube, while in *Cystopus candidus* the resting spores germinate by zoospores. Prillieux (11) has described and illustrated the germination of the oospores of *Plasmopara viticola* (B. and C.) Berl. and De T. He found evidence that the oospores produce a conidiophore. Gregory (6), as a result of more recent investigations, describes and illustrates the germination of the oospores of *Plasmopara viticola* by the formation of a tube or stalk bearing a conidium. This has been confirmed by Ravaz and Verge (12).

Gaümann and Dodge (5, p. 84) have this to say about the germination of the oospores of the Peronosporales: "The germination of the oospores in Plasmopara takes place through zoospores, in *Pl. viticola* under certain conditions also with a germ tube ending in a large conidium (Ravaz and Verge, 1913), in Sclerospora and Peronospora through a germ tube which develops in the host to a mycelium." Apparently, Gaümann and Dodge (5) overlooked Gregory's (6) report made in 1912 of the germination of *Plasmopara viticola*. Gaümann (4, pp. 81, 82) credits his statement on oospore germination of Sclerospora to de Bary, but we have been unable to find where de Bary referred to the germination of Sclerospora. Hiura[3] (7), in a very recent paper (1929), reports in a preliminary way the germination of the oospores of *Sclerospora graminicola* (Sacc.) Schroet. without referring to Gaümann's (4) statement on oospore germination of Sclerospora. Hiura (8) took the oospores from green leaves and placed them in soil water. Under favorable conditions the oospores germinated within 40 to 48 hours and developed hyaline, nonseptate, branched tubes that were three to 11 μ

[1] Published with the approval of the Director of the Iowa Agricultural Experiment Station.

[2] The writers are indebted to Professor I. E. Melhus for suggesting the problem and lending assistance in carrying out the experiments and preparing the manuscript.

[3] Attention is called to a yet more recent publication by Professor Hiura which describes fully a very unique method of germinating the oospores. This appeared during the time the present paper was in press.

Hiura, Makoto. A simple method for the germination of oospores of *Sclerospora graminicola*. Science 72: 95. 1930.

in width. The optimum temperature for germination is reported as between 27° and 30° C. Hiura (7) also refers to a visit to Mr. Da Nami, who showed him germinating Sclerospora oospores and related his method of inducing them to grow. Although the results had not been published, Mr. Hiura's description of Mr. Da Nami's results with germinating oospores leaves no doubt that the oospores were germinating, even though drawings or photographs of the process did not accompany his articles.

When the present work was started in the fall of 1928, the writers were not aware that any one had ever germinated the oospores of Sclerospora. The task of germinating the oospores of *Sclerospora graminicola* seemed hopeful because it was readily possible to bring about infection of *Setaria viridis* (L.) Beauv. seedlings in the soil with the oospores of *Sclerospora graminicola* by the method described by Melhus and Van Haltern (9) and Melhus, Van Haltern, and Bliss (10). This work showed that the oospores germinated and brought about infection of the host within a period of two days. After repeated trials germination of the oospores of *Sclerospora graminicola* was brought about by selecting clean infected tissue in the field in December, 1928, near Ames, Iowa. This was easily obtained by taking the reddish brown shredded tissue from standing Sclerospora-infected *Setaria viridis* plants. The reddish brown color is caused by the abundance of spores formed in the tissues killed by the organism. The oospore material from dry standing plants, as shown in figure 1, usually showed less con-



FIG. 1. A part of a *Setaria viridis* plant killed by *Sclerospora graminicola*. The shredded tissue consists of the fibrovascular bundles left after the pathogene has destroyed the parenchymatous tissues. Among the vascular tissues are abundant reddish brown oospores.

tamination than that collected after the plants had lodged. The oospores were shaken from the diseased tissue into a beaker of sterile distilled water. The spores floated on the surface; however, a few sank to the bottom of the container. From 5 to 30 per cent of the oospores were found to germinate in 24 hours when held at room temperature.

Where clean diseased tissue was not available, the oospores were surface-disinfected with a 5 per cent solution of lactic acid, washed in sterile distilled water, and then floated on sterile water in watch glasses and held at

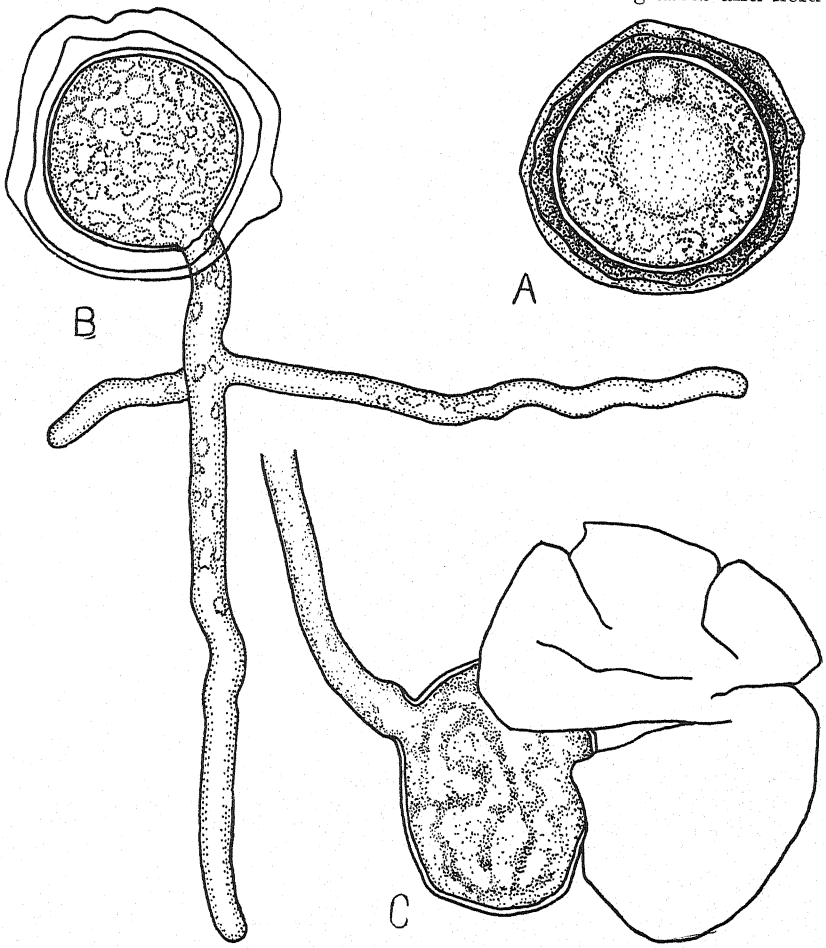


FIG. 2. Oospores of *Sclerospora graminicola*. A. Mature oospore showing exospore, endospore, and the endospore content. B. An oospore sending out a germ tube through a small opening in the exospore. There is direct continuity between the cell wall of the germ tube and the endospore wall. The cytoplasm in the germ tube is hyaline with relatively few granules present. C. The exospore in this oospore has been broken so as to show the exact relationship of the germ tube with the cell wall of the endospore.

18° C. for 24 hours. At the end of this period some of the spores had begun to push out germ tubes. These tubes were hyaline, branched, and contained some globular bodies, as shown in figure 2, A and C. Frequently, long stretches in the tubes seemed to be empty or nearly devoid of cytoplasm, as shown in figure 2, B. The large fat-globule always disappeared before the germ tube developed. Likewise, no evidence of the nucleus was seen in the germinating spores. The granular bodies mentioned were not nuclei. The walls of the tubes were a direct continuation of the endospore wall, as shown clearly in figure 2, C, and they protruded through a small opening in the exospore (Fig. 2, B), apparently dissolved by the action of the forming germ tube. At least, no pore was ever observed in a dormant oospore. The contents of the oospore were frequently seen to flow out of the endospore into the germ tube. In other instances, the contents in the germ tubes were seen to move. The rate of internal activity of the cytoplasm incident to germination under favorable conditions was pronounced. Repeated measurements of germ tubes, after 30 hours at room temperature, were 600 to 700 μ long. The diameter of the tube at the point where it comes out through the exospore wall is smaller in diameter than it is just outside the exospore. When the oospore was severely ruptured this condition was not noticeable. The diameter of the tube was usually about 5 μ. The length and diameter were greater than in many other fungi.

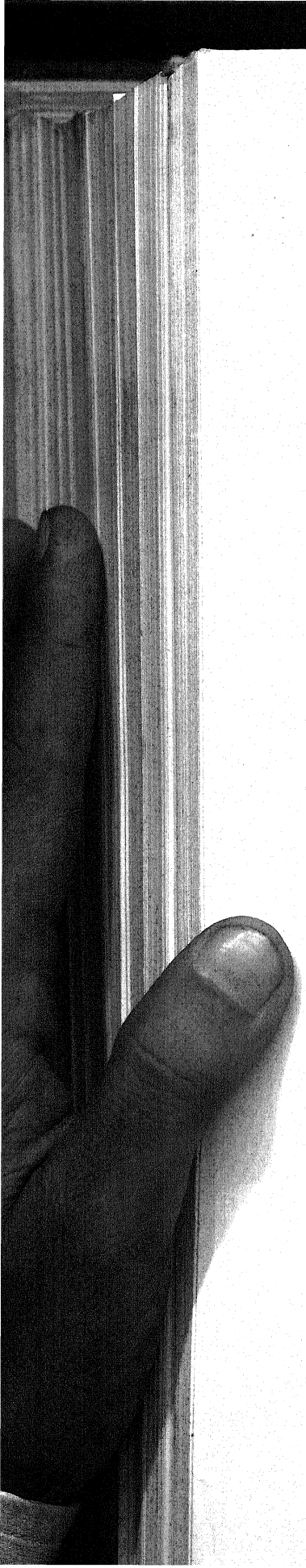
Changes in pH from 4.5 to 7 appeared to have little effect on the germination of the oospores. The oospores which germinated at room temperature branched extensively. The medium used seemed to make little difference; good germination was obtained on agar, malt, potato dextrose, corn-seedling agar, bouillon, and in soil agar. Neither aerial hyphae nor conidia were observed to form on the germ tubes. Occasionally, the ends of the branched tubes were enlarged, but in no case did such enlargement suggest the formation of a sporangium. Just how long these germ tubes would live in media or in water was not determined. The nature of the activity of the cytoplasm in the germinating oospore suggests that the germ tube might continue to live and grow for several days because of the large food reserve in the endosperm.

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DISEASES, NEW TO CITRUS, FOUND IN PALESTINE

I. REICHERT

During 1928 and 1929, a very important period in the development of the Citrus industry in Palestine, there appeared in this country some diseases entirely new to Citrus. Some of them appeared in a very grave form and caused much alarm among local growers. As these diseases are new to science they deserve to be made known to Citrus pathologists in other countries.

LITTLE LEAF

The growth of young orange and grapefruit trees, budded in the fall of 1927, was checked. The branches became stunted, the joints shortened, the leaves smaller, sometimes mottled, and frequently burnt at their tips (Fig. 1, A). The new branches did not bend downwards, as normally, but re-

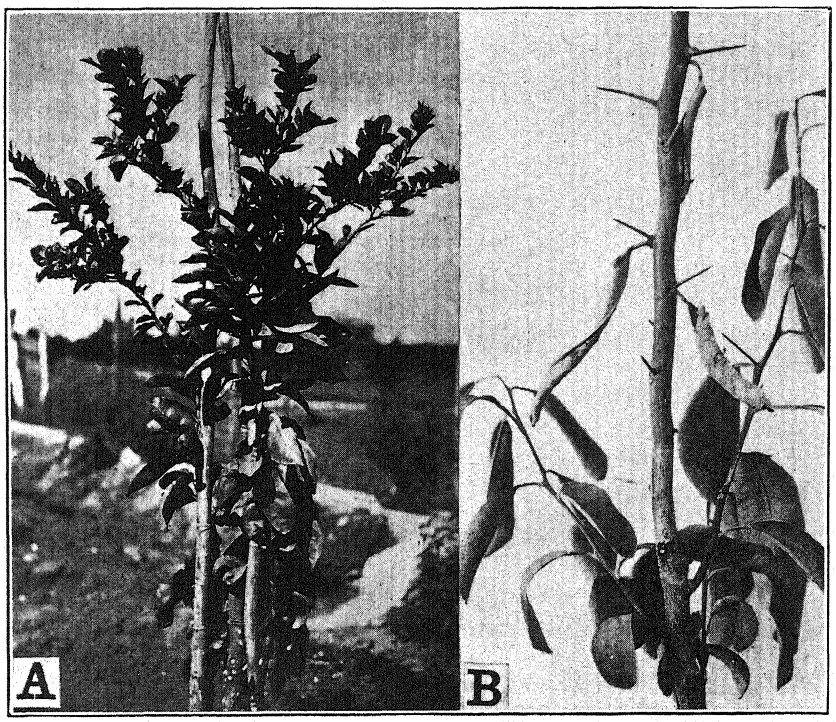


FIG. 1. A. Young orange tree affected with little leaf. (Photograph by J. Perlberger.)
B. Nonbudded sour-orange tree girdled by the spot disease.
(Photograph by F. Littauer.)

mained upright and formed a bush-like growth resembling, to a certain extent, the type of yellows and little-leaf diseases known in other fruit trees (11).

Many suggestions as to the possible cause of the disease, such as over-manuring, overwatering, etc., have been advanced by the growers, but none has been acceptable.

Cultural and histological examination of the diseased parts of the tree have not revealed any positive agent (7). The disease seems to be of a physiological nature, connected with the evaporation of the soil moisture and with the heavy loss of water from the young trees after being cut back. The low rainfall and long hot spells of 1928 caused an unusual desiccation of the young trees. The possible loss of calcium accumulated in terminal parts of trees during the hot spells by the usual cutting back of the stock as well as the pinching back of the young trees may also not be excluded (4).

STEM SPOTS

In the month of May, 1928, there appeared on the western side of non-budded one-year-old seedlings, especially sweet lemon, 10–20 cm. high from the collar, yellow sunken spots which spread and finally girdled the entire stem. As long as the spot was confined to one side of the seedling the branches of the affected side began to yellow and wither. Later on, when the whole stem had been involved, the entire crown withered and died (Fig. 1, B). The affected wood behind the spot had a dark grey appearance. The cortex bore on its surface dots of Alternaria fascicles. The wood vessels behind the spots were found invaded by a mycelium, a pure culture of which has not yet been obtained (8, 10).

Similar spots were found on young trees budded in the previous fall, but no case was known of a spot girdling a budded tree. The necrotic discoloration of the tissue did not go farther than half way up the stem. The wood tissue behind these spots also was found invaded by a mycelium. The damage caused by the second type of spot was particularly great. The disease seems to be of parasitic nature, but some contributing influence of climatic conditions, such as sun, winds, marine humidity prevailing on the western side, etc., must also be taken into consideration.

DYING BACK OF STOCK STUBS

A month after the upper part of the stock of budded trees had been cut back (in April, 1929), the remaining stubs began to die back. The affected part turned brown and the disease spread downwards to the collar. When it reached the bud, the crown began to wither and die back. Sometimes it happened that only the side of the stub opposite the scion had been affected and then the malady spread down to the collar without impairing severely

the growth of the tree. The wood tissue of the necrotic parts showed abundance of mycelium in the vessels. The fungus has not yet been isolated. This disease was found in one case to attack 12 per cent of the trees of a young orchard (8).

SPOTS BELOW THE SCION

This disease appeared especially in 1930. Its characteristic features are: the appearance of brown necrotic spots in the inner bark and wood close under the scion of two-year-old trees. A stunted growth is always associated with the appearance of these spots. The brownish wood tissue is filled up with abundance of fungus hyphae, similarly to the former case.

It is believed that this disease is a later stage of the former one—the dying back of stock stubs. In all these cases the stubs had certainly been affected but unnoticed by the grower who cut them back later. Then the malady spread downwards and caused the brown spots.

STEM ROT

The characteristic symptoms of this disease are the cracking and peeling up of the stem cortex and a discoloration of the wood below it. The under side of the cortex and the upper side of the stem wood are closely covered with numerous sclerotia of *Rhizoctonia bataticola* (Taub.) Butler (9). The rot can be traced down to the roots, where the trouble seems to start.

The association of *Rhizoctonia bataticola* with Citrus stem and root rot hitherto has been reported only from Ceylon (12) where the disease has been found on orange and lime, from the West Indies (2) only on lime, and from Rhodesia (6) only on the orange. Since Ashby (1) and Haigh (5) have shown the existence of 3 strains—A, B, C—in *R. bataticola* characterized by the difference in size of the sclerotia (strain A produces sclerotia of about 1 mm., B of about 200 μ, and C of about 100 μ), it is doubtful whether the strains of *R. bataticola* associated with the Citrus root rots in the above countries are identical. The Palestine strain is in any case not identical with that of Rhodesia. The sclerotia described by Hopkins (6) are of the A-strain type, whereas our fungus belongs to the C strain. Whether the Ceylon and West Indies sclerotia belong also to the C strain has yet to be determined.

Whether *Rhizoctonia bataticola* is the primary cause of collar and root rot of trees, as believed by Small (13), or is of secondary nature, according to Gadd (3), can not yet be said with certainty.

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PHYTOPATHOLOGICAL NOTES

CITRUS DISEASES NEW TO PALESTINE.—In the course of a recent trip through the citrus-growing sections of Palestine the writers encountered the following diseases not previously recorded in Palestine. These were found only in a few places and are not of general distribution.

Exanthema: This disease was found on orange in sandy soil in an orchard not far from the sea. The "stag-horn" appearance of the branches, shortened nodes, multiple buds, curved branches, and stained terminals were evidence of the disease, but the gum pockets usually found on the twigs in connection with the disease elsewhere were absent. This disease is not to be confused with little leaf,[1] which has an entirely different appearance.

Sclerotinia twig blight: This twig blight, caused by *Sclerotinia sclerotiorum* (Lib.) Massee (*Sclerotinia libertiana* Fuckel), was found on a few twigs of lemon trees, *Citrus lemonia.* The dead whitened twigs with formation of sclerotia on the surface indicated the disease. It was not found generally and, up to the present, is of no economic importance in Palestine. The same fungus, however, was doing much damage to bananas in the Jaffa district in 1929–1930, as was found by the Division of Plant Pathology of the P. Z. E. Agr. Exp. St., Tel-Aviv. Oranges inoculated in the laboratory at that time contracted sclerotinia rot. On this basis it was predicted that the disease would soon be found on Citrus in Palestine.

Mal secco: This disease on the twigs, as described by Petri and as distinguished from blast and anthracnose, was found in two orchards on lemon, sour orange, citron (Ethrog), and possibly sweet lemon. The dying back of twigs and the pink discoloration of the wood occurring at considerable distances from the discolored bark and dead end of twigs was like that seen in Sicily. The small-spore Phoma-like fungus was isolated.

Psorosis: This disease, though mentioned once before,[2] is not yet to be considered a serious disease in Palestine. It was identified certainly only in one orchard on Valencia trees. What appeared to be psorosis was also seen in a few old orange groves. Its presence on these trees, however, was somewhat doubtful.—I. REICHERT and H. S. FAWCETT, *Tel-Aviv, Palestine, and Citrus Experiment Station, Riverside, Calif.*

[1] Reichert, I. Palestine Citrograph, 1, No. 11/12, 16–17. 1928.

[2] Ryerson, K. A. The horticultural possibilities of Palestine. Rep. of experts submitted to Joint Palestine Surv. Comm., p. 287. 1928.

Die-back of elm in Minnesota.[1]—During the past two years there has been considerable dying back of elms in Minnesota. The damage seems to be chiefly in nursery stock 10 to 15 feet high, although the disease also occurs in small stock and in larger shade trees. During the summer of 1929 the disease was prevalent throughout the nurseries of the State, suggesting that it may have been present for several years. The number of infected shade trees is yet small, but in some nurseries as high as 25 to 50 per cent of the larger stock is infected.

The causal organism appears to enter through the tips of the branches and works downward through the wood and bark until it reaches the roots. In one shade tree (15 feet high) in St. Paul the fungus had reached the roots and killed the entire tree the second season after the first wilting was observed. The path of the organism through the wood is marked by brownish streaks that may be in a definite ring, as those described for *Graphium ulmi* Schw. in Europe, or, as is more often the case, diffused throughout the wood. The organism finally extends into the cambium and phloem, killing these tissues, but this may not occur until after the leaves have wilted. In many cases the organism appears to invade the cambium and phloem first, causing reddish, sunken cankers.

Isolations made from diseased trees in St. Paul, Minneapolis, Albert Lea, Owatonna, Virginia, Hamel, and Lake City, Minnesota, showed that one fungus was constantly associated with the disease, although other organisms were sometimes present, especially in dead and dried areas. As no fructification of the fungus has been found on elm, positive identification has not been made. In culture, groups of abortive pycnidia containing minute, hyaline, elongate spores are borne in stromata. This places the organism among the Sphaeropsidales, possibly near *Cytosporella*.

About 50 healthy, vigorously growing elms, 2 to 5 feet high, were inoculated during August, 1929, by placing mycelium in agar on freshly exposed wood and covering the inoculum with grafting wax or adhesive tape. When the leaves dropped in the fall no infection had occurred in many of the trees, and in those that became infected the fungus had advanced only 1 to 2 cm. from the point of inoculation. Later, larger trees (1 inch in diameter) were transplanted to the greenhouse and inoculated in a similar manner. Three months after inoculation the trees were dead, the stems being girdled at the point of inoculation and the discoloration extending 5 to 30 cm. from the wound. Transplanting and environmental conditions had noticeably reduced the vigor of these trees. Reisolations showed that the original fungus was present in the diseased areas. Additional inoculations will

[1] Published with the approval of the Director as Paper No. 943 of the Journal Series of the Minnesota Agricultural Experiment Station.

have to be made on vigorous trees in the field in order to determine whether the fungus can kill vigorously growing trees.

Many of the diseased elms in nurseries were in very poor condition, some quite evidently suffering from severe hail and insect injury; others, perhaps from too close cultivation and undercutting, which had reduced the root system severely. In other cases (except for the disease in question), the trees appeared healthy. Observations and inoculations so far made have not shown satisfactorily how virulent the pathogene is and to what extent it will develop in healthy trees. That it is constantly associated with the disease and can kill weakened trees seems, however, to be definitely established. Further investigations are under way.—A. F. VERRALL, *University Farm, St. Paul, Minn.*

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

CONSTITUTION[1]

ARTICLE I

This Society shall be known as The American Phytopathological Society.

ARTICLE II

MEMBERSHIP

Sec. 1. The Society shall consist of members, and may include life members and patrons.

Sec. 2. The charter membership of this Society shall consist of the one hundred and thirty persons who accepted the invitation of the Organization Committee of October 25, 1909, to form the Society.

ARTICLE III

QUALIFICATIONS FOR MEMBERSHIP AND DUES

Sec. 1. All persons interested in the study of phytopathology, including the practical control of plant diseases, shall be eligible to membership.

Sec. 2. Each member shall pay annually such dues as the Society shall determine.

Sec. 3. Any member may become a life member by paying one hundred dollars in ten consecutive annual payments, and any person may become a patron upon the payment of one hundred dollars, and upon election shall have all the privileges of membership.

ARTICLE IV

ELECTION OF MEMBERS

Members may be elected at any regular meeting of the Society or by the Council during the interim. Applications for membership must be endorsed by at least one member of the Society.

ARTICLE V

OFFICERS

The officers of the Society shall consist of a President, Vice President, and Secretary-Treasurer. Their duties shall be those usually performed by such officers. The President and Vice President shall serve for one year and the Secretary-Treasurer for three years, or until their successors are elected, and the Council shall fill any vacancies occurring in the interim between elections.

The Council shall consist of the President, Vice President, Secretary-Treasurer, the retiring President, and the Chairman of the Board of Editors of the Journal of the Society, with two members elected, one each year, who shall serve for a term of two years, and one member elected annually by each Division. The term of service of a Council member from a Division shall commence at the end of the annual meeting of the Society next following his election by the Division.

All action of the Council or officers must be authorized or approved by the Society.

[1] At the 1928 meeting, the incoming and outgoing officers of the Society were directed to prepare a revision of its constitution. This was done, and the proposed changes which were submitted to members one month in advance of the 1929 meeting were later approved by the Society in session at Des Moines. The last printing of the constitution was in PHYTOPATHOLOGY, Vol. 15, No. 10, October, 1925.

ARTICLE VI

ELECTION OF OFFICERS

The Secretary-Treasurer shall send nomination ballots for offices to each member of the Society in time to allow all nominations to be returned not less than two months before the date of the annual meeting. The Council shall make nominations for any office when such nominations are wanting. The three candidates for each office receiving the highest number of nominating votes shall be placed upon a final ballot, which shall be sent to each member one month before the annual meeting. Votes shall be mailed to the Secretary-Treasurer and canvassed by the Council. A plurality vote shall elect.

ARTICLE VII

EDITORS, COMMITTEES, AND APPOINTMENTS

The Editors of the official organ of the Society shall be selected by the Council subject to the approval of the Society.

Temporary or standing committees may be appointed at the discretion of the Society.

Unless otherwise directed, the President shall appoint all temporary committees that are to serve during his administration and shall fill all vacancies on standing committees that may occur during his term of office.

ARTICLE VIII

MEETING

An annual meeting shall be held at such a time and place each year as the Council may select, and additional meetings, including special or local meetings, for the presentation of papers, may be arranged by the Council at its discretion.

ARTICLE IX

DIVISIONS

Branch organizations or units within the Society, known as Divisions, may be established on a geographical basis, provided formal application setting forth the reasons for the establishment of the Division is made to, and approved by, the parent Society.

ARTICLE X

AMENDMENTS

This Constitution may be amended at any annual meeting by a three-fourths majority of all the members voting, notice of the proposed amendment having been sent to all the members at least one month previous to the meeting.

STANDING RULES

The rules under which The American Phytopathological Society operates are as follows:

PHYTOPATHOLOGY

1. The official publication of the Society shall be PHYTOPATHOLOGY.

   a. *Officers.* The officers of PHYTOPATHOLOGY shall be an Editor in Chief, term three years; three Editors, terms three years; twelve Associate Editors, terms three years, four selected each year; Business Manager, term one year; and Advertising Manager, term one year.

   b. *Selection of Officers.* The Editor in Chief and the Business Manager shall be selected by the Council and approved by the Society. The Editors and

the twelve Associate Editors shall be selected by the Council in consultation with the Editor in Chief and approved by the Society. The Advertising Manager shall be selected by the Council in consultation with the Business Manager and approved by the Society.

c. *Subscriptions, Back Numbers.* Subscriptions to PHYTOPATHOLOGY for institutions and nonmembers shall be $6 per year in the United States and dependencies, Mexico, and Cuba; Canada $6.25; other countries $6.50. The price of current single numbers shall be 60 cents. The price of back volumes and numbers shall be determined by the business manager with the approval of the Council. Separate copies will not be sold except in cases where the volumes are already broken. Requests to supply lost copies of the journal without charge must be made within sixty days from date of issue.

DUES

2. The annual dues for each regular member, including subscription to PHYTOPATHOLOGY, shall be $5 per year, payable on December 20. The Business Manager of PHYTOPATHOLOGY shall discontinue sending the journal to any members whose dues have not been received by December 20.

PAPERS, ABSTRACTS

3. Members desiring to present papers at the annual meeting must furnish to the Secretary-Treasurer carefully prepared abstracts presenting as clearly and concisely as possible the substance and conclusions of the papers, these abstracts to embody definite results and not to exceed two hundred words in length.

a. *Date Due.* The Secretary-Treasurer is authorized to refuse abstracts received by him after the date on which they are due (Nov. 15). Members are requested not to submit titles or abstracts unless they intend to be present at the meetings.

b. *Number of.* No member shall be permitted to present more than two papers at any one meeting, except by invitation, and, in case of joint authorship, the paper shall be charged to the author presenting it.

c. *Editing of.* All abstracts shall be submitted to an editorial committee of at least three selected by the Editor in Chief of PHYTOPATHOLOGY, and this committee will edit the abstracts in the same manner as original articles published in PHYTOPATHOLOGY.

PROGRAMS

4. The program for the annual meeting shall be in charge of a program committee consisting of the President, Vice President, and Secretary-Treasurer. To relieve congestion the program committee is authorized to schedule simultaneous sessions when necessary.

DIVISIONS

5. The following provisions shall govern the organization and regulation of Divisions of the Society.

a. *Name of.* Divisions shall use the name of the parent Society with the appropriate geographical term, for example, The American Phytopathological Society, Pacific Division.

b. *Membership.* Divisions shall elect to full membership only members of The American Phytopathological Society, but each Division may elect associate members under such rules as it may adopt.

c. *Publication.* The proceedings of Divisions shall be printed in PHYTOPATHOLOGY. The preliminary abstracts of the Division meetings may, at the discretion of these Divisions, be printed in PHYTOPATHOLOGY under the same rules that govern publication of abstracts of the general Society. This rule, however, shall not be interpreted as limiting the present right of the Editorial Board of PHYTOPATHOLOGY to define the character and amount of any manuscript for publication.

d. *Meetings of.* Whenever The American Phytopathological Society meets within the territory of a Division, the Division shall merge its program with that of the parent Society. The scientific sessions of such a meeting shall be presided over alternately by the President of The American Phytopathological Society and the President of the Division. Business sessions may be independent.

e. *Constitution of.* The constitution or articles of organization of all Divisions shall contain a provision or provisions ratifying the above rules. The constitution of all Divisions shall contain nothing in conflict with the constitution of The American Phytopathological Society. With the exceptions defined by the above rules, the Divisions shall enjoy complete autonomy.

SECRETARY'S EXPENSES

6. Unless otherwise ordered, the Secretary-Treasurer and the President, or, in case of his inability to attend, the Vice President, are authorized to attend the annual meetings of the Society at the Society's expense.

AUDITING COMMITTEE

7. At each annual meeting the President shall appoint an auditing committee to audit the accounts of the Secretary-Treasurer of the Society and the Business Manager of PHYTOPATHOLOGY.

UNION OF BIOLOGICAL SOCIETIES

8. The Society shall participate in the work of the Union of American Biological Societies and designates its Secretary-Treasurer and Editor in Chief of PHYTOPATHOLOGY as its representatives unless otherwise voted.

BIOLOGICAL ABSTRACTS

9. The Society shall provide for a standing committee of five with terms of five years, one member being chosen each year, to cooperate with the Board of Editors of *Biological Abstracts.*

CROP PROTECTION INSTITUTE

10. Representation on the Board of Governors of the Crop Protection Institute shall be provided for by three trustees, with three-year terms, one selected each year, these trustees to be chosen by the Council.

NATIONAL RESEARCH COUNCIL

11. Representation on the Division of Biology and Agriculture of the National Research Council is provided for in the following way. The various societies represented in the Division are classified into groups. This Society and the Society of American Bacteriologists constitute Group V. This group, as is the case with the others, likewise is entitled to one representative. Each Society shall designate an elector and an alternate for three-year terms. The two electors are to choose the representative on the Division for Group V, either one of themselves, or a member of one of the societies, the societies being taken in rotation. The term of the representative shall be three years. The elector from the society not having the representative on the Division in a given period, or some other member whom the Society may select, shall serve as an advisory representative without vote, and without expenses paid by the National Research Council, and may attend divisional meetings to present directly any business of his society.

The elector and alternate of The American Phytopathological Society, terms three years, shall be selected by the Council of the Society and approved by the Society.

OTHER REPRESENTATIVES

12. The following shall be selected by the Council and approved by the Society: two representatives on the Council of American Association for the Advancement of Science for one-year terms; one trustee on the Tropical Plant Research Foundation for a five-year term, and one member of the Editorial Board of the American Journal of Botany for a three-year term.

AMENDMENTS

13. These rules may be amended by a majority vote of the members voting at any regular meeting of the Society.

ANNUAL WINTER MEETING

The American Phytopathological Society will meet in Cleveland, Ohio, during the period Tuesday, December 30, 1930, to Thursday, January 1, 1931, sessions being held at Western Reserve University. Headquarters will be at the Hotel Hollenden. Joint meetings with other organizations are planned as follows: (1) with Section G on Tuesday afternoon, the papers being of general interest, and (2) with the Mycological Section of the Botanical Society of America on Wednesday morning in a de Bary Memorial program. Simultaneous sessions have been scheduled for presentation of a large number of interesting papers dealing with recent developments in research. On Tuesday afternoon provision has been made for a conference on extension work in plant pathology. The annual dinner will be held at 7:00 P. M., Tuesday, December 30, 1930, at a place to be announced later. As usual, details of arrangements will be mailed to all members.

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